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REQUISITES FOR THE RECOGNITION OF THE BLUE-GREEN PSEUDOMONAS

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According to Bergery *et al* (2) organisms of the genus *Pseudomonas* are characterized by the production of a water soluble and diffusible blue-green pigment. In addition 24 of the 31 species listed as belonging to this genus are stated to fluoresce. Hence the property of fluorescence might be also considered as a generic character. Unfortunately both these features are easily overlooked. In many instances, they appear to have been regarded as synonymous terms instead of distinct but not necessarily separate attributes. It seems advisable therefore, to review the factors necessary for their determination.

1. *Pigmentation*

That the production of pigment requires special constituents is not a new observation. On this point the publications since 1880 were reviewed in detail by Georgia and Poe (6) who made a comprehensive study of the organic constituents necessary for the production of pigments by bacteria. In this literature there is general agreement that synthetic media containing asparagine as the source of organic nitrogen, must also contain sulphates, phosphates and probably magnesium.

The writer has found this to hold in the case of the ordinary media used for routine purposes. During the course of some studies on creamery water supply he had occasion to classify a number of organisms. Using media prepared according to "Standard Methods", many of his cultures appeared almost to belong to the genus *Achromobacter* until, by chance, he had to use tap water of lake origin instead of distilled. In consequence an apparent generic metamorphosis took place; most of the supposed *Achromobacters* produced pigment that promptly identified them as members of *Pseudomonas*.

In view of this it is suggested that wider appreciation of the requirements mentioned might lead to organisms of this genus being found more frequently and being considered more significant in certain food products, notably those, such as butter, that are exposed to water borne contamination.

In this regard it is noteworthy that very little attention has been paid to members of this genus other than *Ps. fluorescens*. This species has been incriminated as an important agency in butter deterioration by Jensen (9), Gilruth (8), Eckles (4), Shutt (11), and Morgan (10). In this laboratory,

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many *Pseudomonas* cultures capable of activating decided flavour defects in butter have been isolated from creamery water by the employment of media favourable to their recognition. A number of these may prove to be new species or variants.

2. *Fluorescence*

The literature contains numerous instances of the terms pigmentation and fluorescence being used synonymously but without any intimation as to how the latter is determined.

As defined by Wood (14), "Fluorescence is the property, according to Stoke's Law, possessed by some substances of giving off radiations of longer wave-length than those of the light which excites them. The radiations given off are in the yellow, green and blue colours while the exciting light is the violet or ultra violet." Fluorescence, being a physical property of pseudomonas pigments, therefore would seem to provide a fundamental means for the accurate recognition of an economically important genus.

A method was suggested from the work of Davidson and Gregory (3) who in investigating cases of ring-worm found that violet light produced a diagnostic fluorescence of infected areas of the human scalp.

It seems apparent that recognition of the genus *Pseudomonas* should be based upon:

(1) The use of media suitable for pigment production.
  (a) Nutrient media for routine tests.
  (b) Synthetic media for research purposes.

(2) The demonstration of fluorescence.
  (a) On plates as a means of recognizing *Pseudomonas* colonies.
  (b) In tubes where the fluorescence by pigments might serve as a means of differentiating species or strains of *Pseudomonas*.

Towards establishment of this hypothesis the following experimentation was conducted.

EXPERIMENTAL

1. Media Suitable for Consistent Pigment Production

(a) Nutrient media for routine tests

A review of the literature indicated general agreement that sulphate, phosphate and magnesium are necessary for pigmentation by *Pseudomonas*. From the demonstration of the production of pigment by cultures grown in nutrient broth or agar made with tap water but not in the same media with distilled water it was assumed that this natural water contained these elements.

This assumption is borne out by the statement of Georgia and Poe (6, 7), that the production of fluorescence could be used in place of a chemical test for sulphate, phosphate and magnesium; unless, as also was suggested, the Bacto-peptone was unsuitable for pigmentation. The latter possibility was shown to be improbable because of the use of the same peptone with both waters.

In order to demonstrate that tap water supplied the required inorganic constituents for pigmentation, nutrient broth and agar media were prepared

with distilled water to which SO_4, PO_4 and Mg were added in concentration equal to those in Sullivan's synthetic medium K (12). The comparative pigment producing effects of two peptones were tested also. The specifications of each medium and the results on pigmentation by five *Pseudomonas* and three *Achromabacter* cultures are presented in Table 1. Inoculations were made from cultures grown in nutrient broth made with distilled water. Incubation was at 25° C. for 5 days.

TABLE 1.—COMPARATIVE AMOUNTS OF PIGMENT PRODUCED FROM WATERS, INORGANIC CONSTITUENTS AND PEPTONES IN BROTH AND AGAR MEDIA

Specification of media (Beef extract and reaction of pH 7.2 common to all except Sullivan's "K")	Pigmentation results							
	Test Cultures							
	Ps. 1	*Ps.* 2	*Ps.* 3	*Ps.* 4	*Ps.* 5	*Achr.* 1	*Achr.* 2	*Achr.* 3
Distilled water Bacto peptone broth	−	−	−	−	−	−	−	−
Distilled water proteose peptone broth	−	−	−	−	−	−	−	−
Tap water Bacto peptone broth	+++	++	+++	+++	++	−	−	−
Tap water proteose peptone broth	+++	++	+++	+++	++	−	−	−
Dist. water Bacto peptone broth + 0.2% $MgSO_4$ + 0.1% K_2HPO_4	++	+	++	++	+	−	−	−
Distilled water proteose peptone broth + 0.2% $MgSO_4$ + 0.1% K_2HPO_4	++	+	++	++	+	−	−	−
Distilled water Std. Nutr. agar slants	−	−	−	−	−	−	−	−
Tap water Std. Nutr. agar slants	++	+	++	++	+	−	−	−
Distilled water Std. nutr. agar slants + 0.2% $MgSO_4$ + 0.1% K_2PO_4	+	±	+	+	±	−	−	−
Distilled water synthetic medium "K" as per Sullivan	+++++	++++	++++	++++	++++	−	−	−
Nutr. "K" milk agar in tap water	++++	+++	+++	++++	+++			

Key to test cultures—

Ps, 1 = *Pseudomonas aeruginosa*
Ps. 2 = *Pseudomonas fluorescens*
Ps. 3 = *Pseudomonas mucidolens*
Ps. 4 = *Pseudomonas* species unidentified
Ps. 5 = *Pseudomonas* species unidentified

Achrom. 1 = *Achromobacter putrefaciens*
Achrom. 2 = *Achromobacterl iquefaciens*
Achrom. 3 = *Achromobacter* species unidentified.

The formula for the last medium in Table 1 is given below. This was formulated by the writer for routine research analysis of creamery water. It is a combination of standard nutrient agar and Sullivan's medium "K". The content of skim milk assists in the determination of proteolytic bacteria.

Formula for nutrient "K" milk agar

Bacto-beef extract	0.3%
Bacto-peptone	0.5%
Bacto-asparagine	0.3%
$MgSO_4$	0.1%
K_2HPO_4	0.025%
KH_2PO_4	0.025%
Skim milk	3.0%
Bacto-agar	1.5%
tap-water	
Reaction pH 7.2	

Sterilization—15 pounds steam for 20 min.

RESULTS

Pigment was not produced in any medium made with pure distilled water even with the proteose-peptone, reported by Georgia and Poe (7) to be most favourable for pigmentation. Types of peptones used did not affect differences in results. The addition of SO_4, PO_4 and Mg to distilled water media stimulated pigment production but did not equal that in media made in tap water. Equality might have been secured by more perfect concentrations or balance of the added constituents. This was not attempted. Pigment was less pronounced on the nutrient agar slants than in the liquid media. The synthetic medium and its combination with nutrient agar (nutrient "K" milk agar) gave the most definite pigmentation. This is believed by Sullivan and others to be due, primarily, to asparagine which provides a source of nitrogen more adequate for pigmentation than that of peptone.

(b) Synthetic media for research purposes

The results shown in Table 1 indicate that the synthetic medium favours the production of pigments. Such a medium provides an accurate means for differentiating between organisms of the genera *Pseudomonas* and *Achromobacter*.

Media suggested by Tanner (13), Georgia and Poe (6) were suitable for pigment production. However Sullivan's (12) medium "K" was found the most favourable, and after experimentation this was slightly modified (as shown) to give more outstanding results.

Sullivan's "K" Medium		*Sullivan's "K" Medium (Modified)*	
Asparagine	1.0%	Asparagine	1.0%
$MgSO_4$	0.2%	$MgSO_4$	0.2%
K2HPO4	0.1%	KH_2PO_4	0.05%
distilled water		K_2HPO_4	0.05%
final reaction pH 7.2		distilled water	
		final reaction pH 7.2	

The addition of glycerine favoured more marked differences in the fluorescent colours produced by various cultures the permanency of which required buffering by equal amounts of KH_2PO_4 and K_2HPO_4. The possible use of these colours as a further key in the differentiation of respective cultures will be discussed later.

2. Fluorescence as a Method of Demonstrating Bacterial Pigmentation

For the demonstration of fluorescence in cultures a 400-watt Mazda lamp served as a light source. Except for an area (2 inches square) in which a double thickness of "Corning" No. 587 filters was placed, the bulb was completely enclosed by a generous sheet iron housing. Forced ventilation of the housing was provided by a small "Sirocco" fan. The light emitted was dark purple. It consisted of some extreme red rays but mainly of dark blue and near ultra violet having a dominant wave length of 3650 Å.

RESULTS

In daylight, the results were similar to those of Table 1 in that pigment was formed only from the cultures in media made with tap water or distilled water plus the required inorganic constituents. The pigments varied in intensity from the barely visible to the pronounced. Their colour was pale light green.

Under the violet light, however, these cultures were bright and sparkling. Their colours were yellow, green, greenish blue, blue and dark blue as shown in Table 2. This physical characteristic was unmistakable even in cultures *Ps. 2* and *Ps.* 5, in which pigmentation was scarcely discernible on nutrient agar. All cultures in nutrient media with distilled water and also the *Achromobacter* cultures in all media were of a dull blue fluorescence (designated "?" in Table 2). In this respect they were similar to the uninoculated controls of nutrient media. This is not a pigment fluorescence but due, as reported by Sullivan (12) and Tanner (13) to

Table 2.—Fluorescence of bacterial pigments

Media used (Common reaction pH 7.2)	Fluorescence *(colours)* — Test Cultures								
	Ps. 1	*Ps. 2*	*Ps. 3*	*Ps. 4*	*Ps. 5*	*Ach. 1*	*Ach. 2*	*Ach. 3*	CK.
Distilled H_2O nutr. broth	?	?	?	?	?	?	?	?	?
Tap H_2O Nutr. broth	YG	B	BG	G	GB	?	?	?	?
Distilled H_2O Nutr. broth + 0.2% $MgSO_4$ + 0.1% K_2HPO_4	G	DB	GB	BG	B	?	?	?	?
Distilled H_2O Nutr. Agar slants	?	?	?	?	?	?	?	?	?
Tap H_2O Nutr. Agar slants	G	B	BG	BG	B	?	?	?	?
Synthetic medium K (Sullivan)	YG	B	G	G	BG	O	O	O	O

Legend: Y = yellow
G = green
B = blue
D = dark
– = strong
? = dull blue (fluorescence of medium only)
O = colourless.

peptone exhibiting a weak fluorescence. The synthetic medium was itself non-fluorescent but favourable to the production of pigments which displayed pronounced differences in their fluorescent colours.

(a) Fluorescence of bacterial colonies as a method of recognizing Pseudomonas colonies

When *Pseudomonas* cultures were streaked on agar slants the cultural growth displayed fluorescence under U.V. L, 2 to 3 days in advance of any noticeable pigment diffusion. Similarly, colonies from pure culture platings were easily recognized under the U.V. L. as members of the *Pseudomonas* genus. Some of these colonies did not show pigmentation in daylight even after 5 to 10 days of incubation and accordingly would not have been recognized as *Pseudomonas*.

The use of the U.V.L. for discerning bacteria of this genus, on routine quantitative platings from materials such as creamery water, seems to offer valuable possibilities since these organisms generally are considered detrimental to butter.

However the method presents some limitations for either qualitative or quantitative determinations because only surface colonies display fluorescence. This is due to the fluorescent substance requiring free oxygen for its formation. The piercing or stabbing of all visible subsurface colonies with a sterile needle soon after their appearance, followed by reincubation, in a large measure overcomes the inaccuracies caused by non-fluorescent deep colonies and gives greater applicability to the method.

TABLE 3.—INCREASES IN FLUORESCENT COLONIES FROM WATER DUE TO ADMITTING AIR TO SUB-SURFACE COLONIES

Sample No.	Dilution counted	Colony counts per plate				Increase due to stabbing
		Total	Pigmented in daylight	Fluorescent under U.V.L.		
				Before stab.	After stab.	
	ml.					
19	0.1	11	0	2	5	2.5
20	0.1	65	3	14	27	2.1
21	0.1	14	0	3	7	2.3
22	0.001	128	5	32	75	2.3
25	0.001	130	2	9	17	1.9
27	0.001	47	1	2	6	3.0
31	0.001	52	4	7	19	2.7
35	0.1	129	3	8	25	3.1
36	0.1	160	0	14	37	2.6
12 A.	0.1	72	4	7	22	3.1
						Average 2.56 x

RESULTS

The results secured with this technique, from the creamery water samples, are presented in Table 3. These data indicate the following:

(1) Bacterial colonies must have air or free oxygen to elaborate the fluorescent pigment.

(2) Unless some means of admitting air to sub-surface colonies is provided, accurate determination of *Pseudomonas* organisms cannot be made directly from platings.

(3) The U.V.L. method of recognizing *Pseudomonas* types of colonies seems more accurate and rapid than the usual method which relies upon the observation of pigment diffused into the surrounding medium.

(b) Colours of fluorescence in pigments as a possible means for differentiating species or strains of Pseudomonas

In the modified synthetic medium "K" the differences in colours of fluorescence were so marked as to suggest a possible basic method for demonstrating different species or strains of *Pseudomonas* cultures. The most serious limitation to this characterization is their accurate description.

To formulate some fundamental determination of these colours the following methods have been investigated:

(1) Spectroscopic analysis of pigmented cultures.

(2) The pH of cultures by electrometic methods.

(3) The use of buffered solutions of standard pH.

Each of these methods offers alluring possibilities but also complexities which to date have prevented obtaining conclusive results. Until these are secured it may be stated that there appears to be a direct correlation between the fluorescent colours of the pigments and the pH produced by various cultures. The pigments act as "Colour indicators" varying from yellow-green at high pH values to blue and dark blue at lower ones. If reactions produced by different cultures could be considered a sound characterization, then such cultures might be differentiated by the fluorescent colours which they displayed.

DISCUSSION

With such manuals as: Difco's on *Dehydrated Media and Reagents* (4), and *Standard Methods for the Analysis of Water* and also *Dairy Products* by the American Public Health Association (1) stipulating the use of distilled water for the preparation of media, it may be assumed that many laboratories follow this practice. In consequence it is probable that *Pseudomonas* organisms frequently are unnoticed unless special provision is made for the requirements favouring production of their recognisable character.

In view of this, errors no doubt result in qualitative and determinative bacteriology. This probability is deemed of sufficient importance to justify the inclusion of specific information on media for pigment production in the *Manual of Methods for the Pure Culture Study of Bacteria* by the Society of

American Bacteriologists. It might also be considered for subsequent editions of *Standard Methods for the Examination of Dairy Products* as a result of which such organisms very probably would be found to be of increased significance in the deterioration of these products.

The fluorescence produced by *Pseudomonas* organisms, grown in or on media suitable for pigment production, offers a reliable and practical method of recognizing these bacteria quickly and easily. This can be done directly from platings of any suspected material in a shorter time than that usually required for diffusion of the characteristising pigment. Recognition of sub surface colonies is rendered possible by puncturing the agar above such colonies and thereby allowing the access of air for part of the incubation period.

Colours of fluorescence produced by *Pseudomonas* present a possible additional key to the differentiation of species or their variants. If some fundamental means for qualifying these colours could be established, possible valuable results might be secured. Methods so far investigated indicate that the variety of fluorescent colours is due to the indicator action of the pigment produced by the cultures. If pH of a pigmented culture could be considered a sound characterization, the resulting colour of fluorescence could be used to typify the respective culture.

SUMMARY

1. Nutrient media, made with distilled water, were found inadequate for the production of pigment characterizing *Pseudomonas* bacteria. The same media prepared in tap water favoured pigmentation.

2. The requirements for pigment production were investigated. These proved to be SO_4 , PO_4 and Mg. Tap water supplied them.

3. A nutrient medium, found most efficient for the recognition of *Pseudomonas* and applicable to routine or research analysis, is formulated.

4. A method employing ultra-violet light (U.V.L.) for displaying fluorescence of pigment is presented as a reliable and simple criterion by which to recognize *Pseudomonas* cultures.

5. The variety of colours of fluorescence, exhibited by cultures of *Pseudomonas*, offers a possible method for the differentiation of closely related species or variants.

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A NOTE ON THE CONSUMPTION OF DAIRY PRODUCTS IN THE CITY OF GUELPH, ONTARIO

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A survey was made to determine the dairy product consumption habits of the City of Guelph, Ontario. The population according to the 1931 census is 21,075. To obtain a representative sample, 102 families containing 476 persons were selected at random throughout the city. They were interviewed by the author during the month of October in 1938. Information was obtained under the following four headings:

I. Milk consumed
II. Cream consumed
III. Other dairy products consumed
IV. Consumers' habits.

I.—MILK CONSUMED

For the group as a whole, average daily per capita consumption for all purposes was .74 pt.; the consumption as a beverage was .39 pt.

1. *Consumption of Milk According to Income*

Consumption of milk, both for all purposes and as a beverage, rose with increased income ("on relief," .58 pt.; income $3000 to $4000 per annum, .92 pt.) In the highest income group (over $4000 family income) consumption was lower, however, apparently because of the higher consumption of cream.

2. *Consumption of Milk According to Country of Birth*

Consumption was highest among native-born Canadians (.77 pt.), followed closely by British people (.76 pt.) with Continental Europeans lowest (.63 pt.).

3. *Consumption of Milk According to Occupation*

Consumption increased with the more remunerative occupations. Presence of children in the family, however, was a contributory factor. In the "retired" group the lack of children was probably a contributing factor in showing this group with the lowest consumption.

4. *Adult Consumption*

The average daily per capita consumption as a beverage by all adults was .17 pt. By those actually using milk as a beverage (40.6%) consumption was .70 pt. There was no significant difference in consumption between men and women.

5. *Child Consumption*

The average daily per capita consumption as a beverage by all children was .85 pt.; and by children actually using milk as a beverage 1.13 pt. Consumption was highest in the oldest age group of children (13 to 16

[1] Summary of a fourth-year thesis taken in the Department of Agricultural Economics in 1939.
[2] Regional sales manager at Ottawa, Ontario, of the De Laval Co. Ltd. of Peterborough, Ontario.

years). It decreased as the number of children per family increased. No similar trend was found in the relation of percentage of children drinking milk to number of children in the family.

In the whole group, 29.1% of the children drank beverages other than milk, the percentage decreasing as the income rose (75% in the relief group drank other beverages; no children drank other beverages in the income group above $3000). These beverages included tea, coffee and cocoa; the survey indicated that with a rise in income the amount of milk used in preparing other beverages increased.

II.—CONSUMPTION OF CREAM

The average weekly per capita consumption for the whole group was .12 pt.; 47% of the families used cream, 27.4% using table cream and 19.6% using whipping cream.

1. *Consumption of Cream According to Income*

The use of cream and the use of richer grades increased as income rose, with the sole exception that the "$3000 to $4000" group used less whipping cream than the "$2000 to $3000" group, for which there appeared to be no explanation.

III.—CONSUMPTION OF OTHER DAIRY PRODUCTS.

Weekly per capita consumption of buttermilk (.11 pt.), chocolate milk (.04 pt.), evaporated milk (.55 oz.) and condensed milk (.16 oz.) decreased as income rose.

The lower income groups, instead of using cream in beverages and fluid milk in cooking, substituted canned milk. Buttermilk was more popular as a beverage with low income groups. No family interviewed used milk powder. The number of families assigning a reason for using chocolate milk was too small to permit of any conclusions regarding this product.

IV.—CONSUMER HABITS

Much of the information in this section is believed to be unique, many of the questions never having been reported upon in previous surveys of other municipalities.

1. *Method of Purchase*

In the whole group 46.1% had all milk delivered, 3.9% bought all milk from a store, and 50% bought at both door and store.

2. *Duplication of Delivery*

Chiefly because of sentiment and partly because the brand delivered was not available at the store, 23.6% bought from more than one distributor.

3. *Seasonal Variation in Consumption*

Of the whole group, 16.6% used more milk in winter, chiefly for cooking; 14.7 used more in summer, chiefly as a beverage; 17.6% used more butter in summer, mainly because of melting and for cooking; 11.7% used more butter in winter, mostly for eating.

LIBRARY PURDUE

4. *Consumption of Butter*

In the low income groups, 18.7% reported variation in consumption because of price fluctuations, while in the high income groups only 4.7% reported any variation.

5. *Type of Milk Consumed*

Compulsory pasteurization of all milk sold in Guelph became effective on October 1, 1938, just as the survey commenced. Of the whole group, 80.3% used plain pasteurized milk, and 19.7% used pasteurized-homogenized milk. Of former raw milk consumers interviewed, 22.7% preferred raw milk, and 13.7% preferred pasteurized, while 63.6% noticed no difference; the majority of the latter group were unaware that they had been receiving pasteurized milk since October 1.

6. *Stationary Consumption*

The most important reasons given for failure to use more milk were: "enough purchased" (29.4%), and "high cost" (27.4%). Other reasons included: "did not like milk" (20.5%), and "did not drink milk" (18.6%).

7. *Attitude Toward Delivery Service*

Of the whole group, 85.3% were satisfied with present service and 14.7% offered suggestions for improvement. In the latter group, 40% favoured earlier delivery, 26.6% would have preferred single-service containers, while 13.3% suggested sealed bottles; 6.6% recommended "day delivery in winter," while a similar number were in favour of "uniform delivery time all year."

Since completion of the survey, a municipal by-law has been passed providing for daylight delivery in winter. Some of the local distributors have daylight delivery, throughout the year, while others have night delivery in summer.

8. *Effect of Advertising*

Slightly over 83% of the consumers stated that the present type of advertising of dairy products did not influence the amount consumed. This finding suggests the desirability of co-operative advertising, stressing the benefits of milk generally, in place of the present method of extolling the merits of the particular kinds of milk sold by various distributors.

9. *Effect of Electric Refrigeration on Consumption*

Of those owning electric refrigerators 50% reported an increase in consumption because of coolness of milk used as beverage; the remaining 50% considered refrigeration was only a means of preventing spoilage.

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THE CALYX SPRAY IN CODLING MOTH CONTROL[1]

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Ever since Ball (1) and Melander (7) emphasized the importance of the calyx spray for control of the codling moth, *Carpocapsa pomonella* L., there has been difference of opinion on the subject, particularly among fruit growers. Sometimes in commercial operations the calyx spray is omitted with apparently no increase in codling moth injury. Again it may be omitted with apparently serious results. In some years relatively few of the total entries are made at the calyx end of the apple; in other years there are many such entries.

In the Okanagan Valley of British Columbia, there is much interest in the role of the calyx spray in codling moth control. Previous to 1939 the British Columbia Department of Agriculture published several reports dealing with the omission of the calyx spray. In 1939 and again in 1940 that department conducted calyx spray investigations involving about three acres in replicated blocks each season. At the same time the Dominion Division of Entomology undertook to investigate the problem in the laboratory. This paper deals chiefly with the laboratory investigations.

FIELD RECORDS OF THE BRITISH COLUMBIA DEPARTMENT OF AGRICULTURE

The records of the British Columbia Department of Agriculture concerning the omission of the calyx spray in field experiments have been brought together in Table 1. The data from 1932 to 1937 are from the Annual Reports of that Department. Those of 1939 and 1940 are taken from reports to the Western Co-operative Spray Project, and are quoted by permission of B. Hoy, in charge of the investigations.

TABLE 1.—FIELD RECORDS OF CODLING MOTH INFESTATION, KELOWNA, B.C.

Year	Wormy fruit	
	Calyx sprayed	Not calyx sprayed
	%	%
1932	1.4	3.6
1933	0.0	0.3
1935	0.5	0.7
1937	6.5	8.4
1939	6.4	7.7
1940	6.9	9.9

[1] Contribution No. 2110, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.
[2] Agriculture Assistant.
[3] Officer-in-Charge.

Apparently under the conditions of the experiments, *i.e.*, thorough spraying with adequate equipment, the application of the calyx spray resulted in lower codling moth infestation. Although the difference in favour of the calyx treatment was in no case pronounced, it was consistent from year to year.

EXPERIMENTAL PROCEDURE FOR LABORATORY INVESTIGATIONS

Season of 1939

Eight 20-year-old McIntosh trees were sprayed with lead arsenate 3.75 pounds, casein-lime spreader 3.3 ounces, water 100 Imperial gallons, at the "calyx" period, *i.e.*, when about 90% of the petals had fallen. Four of the trees were sprayed with a "Hardie Model E" spray gun from the ground only. The disc aperture was 7/64-inch, the estimated pressure at the gun was 325 pounds, and the output was 4 Imperial gallons per minute. The trees, about 15 feet high, received approximately 15 Imperial gallons each. Four of the trees were sprayed with an angle nozzle on a bamboo rod, with a disc aperture of 4/64-inch and an output of $1\frac{1}{3}$ gallons per minute. These trees were sprayed from the top of the spray tank as well as from the ground, every effort being made to force the spray into the calyx cups. Two trees were not sprayed and served as checks.

Season of 1940

Four 20-year-old McIntosh trees were sprayed at the calyx period with the same spray mixture as used in 1939. A "Hardie Model E" gun was used, with a disc aperture of 8/64-inch and pressure of about 400 pounds at the gun. Spraying was from the ground only. Two check trees were not sprayed.

In neither season was any spray applied but the calyx treatment. Fruits were sampled from time to time beginning about a month after the spray had been applied. The samples were removed to the laboratory and individual freshly-hatched codling moth larvae placed upon the sepals. Small vials, 20 × 5 mm. inside measurement, were then placed over the calyx parts and sealed in position with warm paraffin wax. Eight to ten days later the fruits were dissected and larval activities recorded.

Other samples were selected for chemical analysis by M. P. Trumpour, Division of Chemistry, Dominion Department of Agriculture, Summerland, B.C.

RESULTS OF LABORATORY EXPERIMENTS

A. *Arsenical Residues*

Analyses of the calyx parts indicated that traces of arsenic may be present on fruit from unsprayed trees as far as 100 feet away from sprayed trees. Table 2 gives the average results from quadruplicate determinations within which the maximum variations were less than 15%. The results in 1939 were from sepals, stamens, and pistils only, whereas those in 1940 were from discs removed from the calyx of the apple with a cork-borer and included portions of the skin of the fruit immediately adjoining the bases of the sepals. This was the area enclosed by the glass vial used to confine the codling moth larva to the calyx parts.

TABLE 2.—ARSENICAL RESIDUES ON CALYX PARTS OF APPLES

Date sprayed	Date sampled	Type of nozzle	Sampled at	As_2O_3 per 10 calyx cups
			Feet	Micrograms
May 12/39	Aug. 11-21/39	Hardie gun	6–8	128
May 12/39	Aug. 11-21/39	Hardie gun	15	80
May 12/39	Aug. 11-21/39	Angle nozzle (bamboo rod)	6–8	101
May 12/39	Aug. 11-21/39	Angle nozzle (bamboo rod)	15	90
May 12/39	Aug. 11-21/39	Not sprayed	6–8	Trace
May 14/40	July 17-Aug. 16/40	Hardie gun	6–8	214
May 14/40	July 17-Aug. 16/40	Not sprayed	6–8	14

These determinations indicate that the use of a spray gun from the ground only results in spray deposits on the calyx parts fairly comparable to those obtained from a rod with angle nozzle used from both ground and top of spray tank. Although it required three times as long to spray a tree with the rod-angle nozzle as with the spray gun, the amount of spray applied was approximately the same.

B. *Experiments with Larvae*

Results from the application of individual larvae to the calyx parts of fruits calyx-sprayed and not sprayed are summarized in Table 3. The calyx spray was applied May 14, with a spray gun. Arsenical analyses from representative samples of the fruits are given in the previous table.

TABLE 3.—MORTALITY OF FIRST INSTAR CODLING MOTH LARVAE ON CALYX PARTS OF APPLES

——	Total larvae	Entered through calyx	Entered at outer base of sepals	Total successful entries	Mortality
		%	%	%	%
Calyx sprayed fruit	296	3	14	17	83
Fruit not sprayed	298	28	44	72	28

These figures cannot be related directly to field conditions because, aside from the fact that the experiments were carried on at fairly uniform temperature in the laboratory (70 to 77° F.), the enclosure of the calyx by a small glass vial resulted in a decidedly artificial environment. But in so far as the experiments indicate the capacity of the codling moth larva to penetrate an arsenical deposit on the calyx parts, they have a bearing on codling moth control.

It is interesting to find that, although restricted closely to the calyx parts, only 3% of the larvae succeeded in establishing themselves within the calyx on fruits that had received a calyx spray applied by a spray gun; 28% established themselves within the calyx on fruits not sprayed.

Another interesting point is that on calyx-sprayed fruit only 14% of the larvae succeeded in establishing themselves by attacking the outer base of the sepals as compared to 44% on fruits not sprayed. This difference suggests that the calyx application resulted in deposition of poison on the outer surfaces and outer bases of the sepals as well as the inner surfaces, although in some instances the sepals were so far folded back it seemed doubtful if their outer (under) surfaces would be hit by the spray.

Over a period extending from one to three months after the application of the calyx spray, the total mortality on sprayed fruits was 83% and on fruits not sprayed 28%. One month after the calyx spray had been applied, mortality on sprayed fruits was 87%, and on fruits not sprayed 28%. Three months after the calyx treatment the respective values were 72% and 15%. These data reveal that a lead arsenate deposit resulting from a calyx spray may retain its effectiveness to a high degree for several months after application.

DISCUSSION

Laboratory investigation with first instar codling moth larvae confined individually to an area five millimeters in diameter in the calyx basin of developing apples, has indicated that a calyx spray of lead arsenate applied when 90% of petals have fallen, has a measurable effect in preventing entries through or adjacent to the sepals.

Wiesmann (8) observing the behaviour of codling moth larvae in the orchard, came to the conclusion that in a one-generation area at least, the calyx spray was effective not so much by virtue of the amount of poison deposited within the inner calyx cup as by the deposition of poison on the sepals. He stated that the larvae in chewing about the calyx parts, attack the sepals, not the stamens or pistil. The present investigation confirmed Wiesmann's conclusions. The observations in both instances deal with larvae attacking the fruit several weeks after the petals have fallen. Earlier attacks might show different behaviour, for the calyx parts would then be in a more succulent condition.

Since in these experiments the great majority of the larvae did not penetrate directly into the inner calyx cavity by chewing either stamens or pistil but did chew freely on the sepals, it might be assumed that an arsenical deposit on the sepals would be more likely to be ingested than a deposit on the inner floral parts. To judge from the records of mortality this actually was so.

It has been maintained by Ball (1) and Melander (7) that to be effective the calyx spray must be forced into the inner cavity of the calyx, that is, the cavity roofed over by the ring of stamen filaments. But with the most common varieties at least, these filaments are so closely in contact with one another it seems improbable that this end can be attained with present-day equipment. Despite this apparent barrier, however, over 80% of the inner calyx cavities of McIntosh apples that received a calyx spray applied with a single nozzle spray gun were found to contain particles of spray residue readily visible with low magnification. In this instance the disc aperture of the spray gun was 7/64-inch and the pressure at the nozzle approximately 400 pounds. Possibly surface forces play an active role in the movement of spray liquid through the interstices of the stamen ring.

Observations in the West for a number of years indicate that so-called calyx entries in the field very frequently are not calyx entries in the strict sense, *i.e.*, entries through the walls of the inner calyx cavity, but rather entries at the base of the sepals. Accordingly a deposit of poison within the inner cavity of the calyx would have relatively little opportunity of becoming effective.

It has been noted by Garlick (6) that the earlier investigators in codling moth control estimated 75% of first generation larvae made calyx entries (assumed to indicate merely entries at the calyx end of the fruit). He has found that in Ontario the percentage of calyx entries is considerably less than this, and he speculates upon the possibility of changing habits in the codling moth larva. In the West, calyx entries appear as a rule to represent an even smaller percentage of the total than in Ontario.

In most orchards there are varieties of apple that vary considerably as to period of calyx closure. It seems manifestly impossible under practical conditions to apply to such orchards in their entirety the calyx spray of Ball and Melander. Nevertheless it has been demonstrated in the field that as a rule a calyx treatment so-called, when most of the petals have fallen, may be expected to exert a measurable effect on codling moth infestation, and this even with equipment adequate to ensure rapid and timely application of subsequent sprays.

It appears probable that the calyx spray as applied by a spray gun gives protection chiefly by deposition of poison on the sepals, rather than on the stamens or pistil or within the inner calyx cavity. If this is so, the chief functions of the calyx spray under British Columbia conditions would then be: first, to ensure a poison coverage on leaves and developing fruits, including the inner surfaces of the sepals, in advance of the earliest larvae; second, to act as a "foundation" for later deposits so that maximum protection is provided during the most critical days of first brood attack.

Since most British Columbia orchardists for one reason or another cannot be reasonably certain of completing a spray treatment in less than about 10 days, and since codling moth attack may sometimes commence less than two weeks after petal fall, the application of a calyx spray can be regarded as a sound and conservative procedure in British Columbia. But there appears to be no reason to insist that in applying what is now known as the calyx spray, it is absolutely necessary to force the spray liquid into the innermost recesses of the calyx structure.

SUMMARY

1. In laboratory investigations, first instar codling moth larvae when penetrating the calyx cavity a month or more subsequent to petal fall, chewed very little on the inner parts of the calyx, but did so fairly extensively on the sepals.

2. A calyx spray of lead arsenate applied by a spray gun when 90% of the petals had fallen was found to be fairly effective in preventing the entry of codling moth larvae at the calyx a month later. Three months after application the calyx treatment still showed distinct value.

3. An angle nozzle on a bamboo rod, used from spray tank as well as ground, deposited no more spray on the calyx parts than a single nozzle spray gun used from the ground alone, although the operation required three times as long.

4. The functions of the calyx spray as now applied in British Columbia appear to be to deposit poison on the inner surfaces of the sepals, to ensure protection before the earliest first generation larvae are hatched, and to act as a basis for later sprays and so contribute to a maximum deposit when attack of first generation larvae becomes general.

5. Application of the calyx spray for codling moth control in British Columbia appears to be sound practice, though there seems no reason to believe that in applying that spray it is essential to force spray material into the inner calyx cavity.

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PREVALENCE OF SEED-BORNE FUNGI ON CEREALS IN CERTAIN SEED INSPECTION DISTRICTS OF CANADA[1]

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An investigation was carried out in 1939 and 1940 to obtain more definite information regarding the relative prevalence, distribution, and economic importance of seed-borne diseases of small grain crops in Canada. As pointed out by Craigie (3) in 1937, seed-borne diseases exact a heavy annual toll from every important farm crop in this country. In the investigation a large number of seed samples of wheat, oats, and barley were examined for disease. Approximately one-half of the samples were provided by the Plant Products Division, Production Service, Dominion Department of Agriculture. Although various technical problems were involved in the work, only those results of particular interest to seed growers, seed tradesmen, seed analysts, and seed inspectors are presented at this time. Some of the practical aspects of the seed-borne disease problem given especial attention are (1) disease-producing fungi associated with the seed of cereal crops, (2) the relation of seed infection to seed germination and to the occurrence of seedling blight, rootrot, and certain important leaf-spotting diseases of cereals, and (3) the control of seed-borne diseases by seed treatment. A detailed description and discussion of the pathological analysis methods used in the investigation will be given in a later paper.

MATERIALS AND METHODS

In the fall of 1939 more than 1,800 seed samples of wheat, oats, barley and rye, from all provinces, were submitted by the Plant Products Division to the Winnipeg Laboratory for examination. They consisted chiefly of Elite, Registered, and Certified seed stocks. Approximately 1,100 of them were examined critically for seed-borne diseases. In addition, the health condition of 560 seed samples of wheat, oats, and barley from Manitoba, and of more than 500 samples of wheat from Saskatchewan and Alberta was determined. The Manitoba samples were obtained from some 330 farms and represented farmers' seed stocks for 1940, while the Saskatchewan and Alberta grain samples were of carload lots which were obtained through the courtesy of the Grain Inspection Division, Department of Trade and Commerce, Winnipeg, Man. In 1940, the Plant Products Division submitted 821 samples of wheat, oats and barley, all of which originated in Manitoba and the more eastern provinces. In addition, 390 seed samples of wheat, 218 samples of barley, and 127 oat samples, collected from more than 400 farms in Manitoba, were examined. The distribution by crops and by seed-inspection districts of the samples received from the Plant Products Division, and from the other sources, is given in Table 1.

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TABLE 1.—NUMBER OF SEED SAMPLES OF WHEAT, OATS, BARLEY AND RYE FROM THE 1939 AND 1940 CROPS, AND FROM DIFFERENT SEED INSPECTION DISTRICTS IN CANADA, EXAMINED FOR SEED-BORNE DISEASES

Origin of seed and year produced (Seed Inspection Districts)	Wheat	Oats	Barley	Rye	Total
1939					
Maritime Provinces	20	62	29	0	101
Quebec	7	41	24	1	73
Eastern Ontario	10	1	2	0	13
Western Ontario	56	7	3	0	66
Manitoba	341	89	141	1	582
Saskatchewan	505	28	27	11	571
Alberta	442	150	102	0	694
British Columbia	17	44	21	2	84
Total	1,398	422	349	15	2,184
1940					
Maritime Provinces	1	19	4	0	24
Quebec	3	29	15	0	47
Eastern Ontario	11	195	64	0	270
Western Ontario	16	237	76	0	329
Manitoba	455	170	261	0	886
Total	486	650	420	0	1,556

The methods used for the detection of seed-borne diseases have been described in considerable detail by Doyer (4, 5), Crosier (2) and Porter (14), and a synopsis of the pathological methods used in seed testing practice has recently been published by Neergaard (12). As has been pointed out by these workers, the technique to be adopted in determining the disease condition of seed depends upon the organism and host concerned. Obviously, the technique required to discover the presence of one seed-borne disease may not be at all suitable for the detection of another disease carried by the same seed. As each disease requires a particular technique for its detection, further investigation is still necessary before completely satisfactory methods of seed pathological analysis are devised for any given crop.

Owing to the fact that the number of grain samples submitted for examination from the 1939 and 1940 crops was large, no elaborate or time-consuming methods could be employed. Previous experience with cereal seed samples of the 1937 and 1938 crops in Manitoba had revealed that, apart from the determination of the amount of smut on the seed, the health condition of grain samples could be satisfactorily determined by employing two different techniques, namely, a laboratory agar-plate test and a greenhouse soil-bed test.

The agar-plate method used was that developed and employed by the writers in earlier studies with cereal seeds (10), and consists essentially in plating out 100 surface-sterilized seeds of each sample on potato dextrose agar in Petri dishes. The plate cultures are then incubated at from 24° to 26° C. for 7 days, and, at the end of this period, the organisms growing

out from the seed are identified, or isolated for further study. This method is valuable in mycological research, but as it furnishes only part of the information that is required, it serves only as an auxiliary or complementary method in seed-borne disease investigations.

The soil test consists of growing seedlings from treated and untreated lots of each grain sample in beds of ordinary soil in the greenhouse. A mixture of New Improved Ceresan and talc is used as the seed disinfectant. About 10 days after planting the following data may be obtained: Percentage germination, number of retarded, stunted, and weak seedlings, the percentage of diseased seedlings, and the degree of seedling infection caused by the various species of root-rotting and leaf-spotting fungi borne in and on the seed.

As all of the samples could not be tested at one time, the soil and seed treatment techniques were carefully standardized, and individual tests were carried out under soil temperature and moisture conditions favourable for the infection of seedlings with the fungi found on the seed, but as unfavourable as possible for infection arising from certain common soil-inhabiting fungi, such as *Rhizoctonia solani* and species of *Penicillium*. Check samples were included in each test, and the occurrence of any variation between tests was recorded. The soil test proved very satisfactory for cereals. It gave an accurate measure of the amount of damage caused by surface-borne as well as by internally-borne seed organisms, excepting the smuts, and indicated the presence or absence of non-parasitic seed troubles. In addition, it provided reliable information concerning the germinability of the seed, and showed to what extent germination and disease development were affected by seed treatment.

No attempt was made to examine the 1939 seed samples for surface-borne smuts, although all the 1940 samples were examined for these diseases. This examination was effected by washing the seed, and then centrifuging and examining the surface washings for the presence of smut spores. In the present investigation, a spore load equivalent to 1 part of smut to 128,000 parts of grain, by weight, was considered sufficiently heavy to justify seed treatment. The actual relation of the spore load, as determined in the laboratory, to the occurrence of smutted plants in the field requires further study. Laboratory and field experiments to determine this relationship are now under way.

EXPERIMENTAL RESULTS

Disease-producing Fungi Associated with Wheat, Oats, Barley and Rye Seed

As is well known, many different kinds of organisms may be carried by cereal seed (13), but by far the most prevalent and destructive ones are the fungi. Bacteria are commonly associated with such seed, although comparatively few pathogenic forms have been found. Species of fungi belonging to more than 50 genera, and many different kinds of bacteria were isolated from the seed samples of 1939 and 1940. Among the fungi isolated, species of *Alternaria* were by far the most common, and next in order of importance were species of *Helminthosporium* and *Fusarium*. Extensive pathogenicity tests have shown, however, that species of *Alter-*

naria are not the principal cause of seedling blight, rootrot, and leaf-spotting diseases in cereals. On the other hand, it has been repeatedly demonstrated that species of *Helminthosporium* and *Fusarium* are the important pathogenic fungi associated with the seed of small grain crops, and hence the results in this paper deal mostly with them.

TABLE 2.—THE PREVALENCE OF DISEASE-PRODUCING AND OTHER FUNGI IN SEED SAMPLES OF WHEAT AND BARLEY FROM THE 1939 AND 1940 CROPS, AND FROM DIFFERENT SEED INSPECTION DISTRICTS

Origin of seed and year produced	Number of samples examined	Percentage of seeds infected with:[1]						
		Alt.[2] spp.	*Hel.*[3] *sativum*	*Hel.* spp.	*Fus.*[4] spp.	Other[5] fungi	Bacteria	Clean seeds
		Wheat						
1939								
Maritime Provinces	20	46	5.5	0.5	4.1	10.7	2.1	34
Quebec	7	65	0.1	0.0	2.9	7.7	2.7	26
Eastern Ontario	10	58	2.7	0.3	1.8	2.0	20.3	30
Western Ontario	53	26	0.5	0.1	0.2	1.0	0.4	72
Manitoba	541	56	2.3	0.6	0.4	4.0	1.5	41
Saskatchewan	406	16	0.2	0.1	0.1	0.6	0.3	83
Alberta	223	5	0.02	0.0	0.1	2.3	0.5	92
British Columbia	17	7	0.0	0.0	0.1	3.0	1.4	89
1940								
Maritime Provinces	1	75	0.0	0.0	0.0	3.0	0.0	22
Quebec	3	61	0.7	0.3	2.3	11.0	0.0	29
Eastern Ontario	11	83	3.8	0.2	0.2	3.0	0.0	13
Western Ontario	16	70	0.4	0.1	0.2	0.9	0.6	29
Manitoba	455	72	2.8	0.3	0.9	2.1	2.4	24
		Barley						
1939				*Hel.*[3] *teres.*				
Maritime Provinces	29	49	35.4	3.6	4.4	14.9	2.4	6
Quebec	24	73	11.4	4.1	6.7	5.0	7.5	6
Eastern Ontario	2	78	0.5	0.5	2.0	4.0	3.0	11
Western Ontario	3	89	3.3	0.3	1.0	5.0	6.0	7
Manitoba	141	60	2.7	0.4	0.9	6.0	5.5	29
Saskatchewan	27	26	0.2	2.0	0.1	5.4	4.3	66
Alberta	102	12	0.2	0.6	0.3	10.3	1.7	76
British Columbia	21	11	0.1	1.2	0.4	10.1	2.0	77
1940								
Maritime Provinces	4	77	11.7	0.8	0.5	6.5	0.0	15
Quebec	15	83	0.7	0.1	0.1	2.4	0.1	11
Eastern Ontario	64	80	4.4	0.2	3.4	7.5	0.5	11
Western Ontario	76	88	5.4	0.03	1.7	4.3	0.3	7
Manitoba	261	82	3.4	3.1	1.4	3.2	4.1	12

1 Based on plating out 100 surface-sterilized seeds of each sample on potato dextrose agar. Data are means of district samples.

2 *Alt.* = *Alternaria.*

3 *Hel.* = *Helminthosporium.*

4 *Fus.* = *Fusarium.*

5 Chiefly fungi belonging to the genera *Nigrospora, Curvularia, Hormodendron, Epicoccum, Septoria Cephalothecium, Macrosporium,* and *Penicillium.*

The results of the plating tests are summarized in Tables 2 and 3. As these tables show, *Helminthosporium sativum* P.K. and B., *H. teres* Sacc., and *H. Avenae* Eidam were the predominant species of *Helminthosporium* isolated from surface-sterilized seeds of wheat, oats, barley and rye. The seedling blight and root-rotting fungus, *H. sativum*, was commonly associated with wheat, barley, and rye seed, while *H. teres*, the fungus causing net blotch of barley, was frequently isolated from barley seed. On the other hand, *H. Avenae*, the fungus causing the leaf blotch disease of oats, was by far the most common species of *Helminthosporium* isolated from oat seed. This fungus was frequently obtained from samples produced in the Maritime Provinces, Quebec, and Ontario, but only occasionally was it isolated from oat seed produced in the Prairie Provinces and British Columbia. In passing, it may be mentioned that *H. sativum* was frequently isolated from oats, and *H. teres* occasionally from oat and wheat kernels.

Many different species of *Fusarium* were isolated from surface-sterilized cereal seed, but *Fusarium poae* (Pk.) Wr., *F. avenaceum* (Fr.) Sacc., *F. scirpi* Lamb. and Fautr. v. *acuminatum* (Ell. & Ev.) Wr., and *F. Equiseti* (Cda.)

TABLE 3.—THE PREVALENCE OF DISEASE-PRODUCING AND OTHER FUNGI IN SEED SAMPLES OF OATS AND RYE FROM THE 1939 AND 1940 CROPS AND FROM DIFFERENT SEED INSPECTION DISTRICTS

Origin of seed and year produced	Number of samples examined	Percentage of seeds infected with:[1]						
		Alt.[2] spp.	*Hel.*[3] *sativum*	*Hel. Avenae*	*Fus.*[4] spp.	Other[5] fungi	Bacteria	Clean seeds
		Oats						
1939								
Maritime Provinces	10	37	0.4	9.4	7.5	32.5	2.0	22
Quebec	6	57	0.02	4.5	8.9	12.2	2.0	26
Western Ontario	7	57	1.6	1.3	5.4	6.0	2.3	31
Manitoba	19	36	0.7	0.8	0.9	6.1	1.9	57
Saskatchewan	28	13	0.3	0.7	0.2	8.4	0.5	78
Alberta	10	4	0.0	0.0	0.0	8.2	1.3	87
British Columbia	17	8	0.0	0.4	1.0	18.1	0.5	74
1940								
Maritime Provinces	19	42	0.5	7.2	6.3	13.8	0.3	34
Quebec	29	56	0.5	6.8	8.4	8.3	0.8	30
Eastern Ontario	100	61	0.2	5.6	5.1	8.2	0.7	28
Western Ontario	100	69	1.1	4.8	3.7	6.7	1.3	23
Manitoba	170	50	1.2	0.6	1.0	4.0	3.6	45
		Rye						
1939								
Quebec	1	39	1.0	0	3.0	16.0	12.0	30
Manitoba	1	49	1.0	0	1.0	3.0	7.0	39
Saskatchewan	11	33	0.5	0	0.0	3.6	3.1	61
British Columbia	2	16	0.0	0	0.0	10.5	1.5	73

[1] Based on plating out 100 surface-sterilized seeds of each sample on potato dextrose agar. Data are means of district samples.
[2] *Alt.* = *Alternaria.*
[3] *Hel.* = *Helminthosporium.*
[4] *Fus.* = *Fusarium.*
[5] Chiefly fungi belonging to the genera *Nigrospora, Curvularia, Hormodendron, Epicoccum, Septoria, Cephalothecium, Macrosporium,* and *Penicillium.*

Sacc. were the predominant ones. Recently, however, Johnston (6) found that most of these fungi are either non-pathogenic or only mildly pathogenic to wheat seedlings. The seedling-blight fungus *Fusarium culmorum* (W. G. Sm.) Sacc. was only occasionally isolated from cereal seed. The species of *Fusarium* isolated from the seed of the 1939 and 1940 harvests have been identified by Dr. W. L. Gordon, of this Laboratory.

Both in the 1939 and 1940 seed samples, species of *Helminthosporium* were more frequently isolated than were those of *Fusarium*. It was observed that kernels infected with *Helminthosporium* species were often infected with a species of *Fusarium* also, as well as with certain other fungi. Individual kernels in some samples were infected with as many as five different species of fungi, and with bacteria.

In these two years, as might be expected, the amounts of seed infection within the same seed inspection districts showed some variation. For example, oat and barley seed in the eastern provinces carried less infection in 1940 than in 1939, although, even in 1940, a fairly heavy infection with *Helminthosporium sativum* and *Fusarium* species occurred in the Maritime Provinces and Quebec. Because of a more severe infection with *Alternaria*, certain other fungi and bacteria, the total amount of wheat, oat, and barley seed infection was greater in Manitoba in 1940 than it was in 1939.

Each year, there were marked differences in the prevalence of species of *Helminthosporium* and *Fusarium* in wheat, oats, and barley seed produced in different seed inspection districts, which, for the purposes of this paper, may be regarded as provinces. For instance, wheat produced in the Maritime Provinces in 1939 carried with it, to a relatively dangerous degree in most samples examined, organisms that are responsible for seedling blight, rootrot, and leaf spot, while only a small percentage of the seeds in samples of wheat from the Prairie Provinces and British Columbia was infected with these disease-producing organisms. Samples of rye obtained from Saskatchewan and British Columbia in 1939 were comparatively free of fungi and bacteria, 61% and 71% of the kernels, respectively, yielding no organism when plated out on agar in Petri dishes. In 1939, the average percentage of barley seeds infected with *Helminthosporium sativum* in 29 samples from the Maritime Provinces was 35.4%, while in 102 samples from Alberta it was only 0.2%. In the same year, the average percentage of barley seeds infected with species of *Fusarium* in 24 Quebec samples was 6.7%, while in 141 Manitoba barley samples less than 1% of the seed was infected. In both 1939 and 1940, the eastern provinces produced oat seed that was far more heavily infected with *Helminthosporium Avenae* and species of *Fusarium* than was seed produced in Manitoba, and in the other western provinces.

Furthermore, there were marked differences even within districts between samples in the percentage of seeds infected with disease-producing fungi. In 1939, for instance, the percentage of seeds infected with *H.sativum* in samples of barley from the Maritime Provinces varied from 1 to 98%, and with species of *Fusarium* from 0 to 16%. As a matter of fact, it was often found that samples of wheat from different fields on the same farm often varied greatly with respect to the percentage of seeds infected with

pathogenic, and other fungi. The present studies show how widely seed infection varies in Canada from year to year, from province to province, and even from field to field.

The Relation of Species of *Helminthosporium* and *Fusarium* in Wheat, Oats, and Barley Seed to Seedling Blight, and Other Diseases

From the point of view of the grower, the relationship between organisms borne in and on cereal seed and the occurrence of disease in the young plants arising from the seed is important. In 1932–34, Christensen and Stakman (1) examined a large number of samples of barley produced in Minnesota and other northwestern States for disease-producing organisms, and found that there was a high correlation between the percentage of seed infected with *Helminthosporium* and *Fusarium* and the percentage germination of the seed, amount of rootrot, seedling blight, the number of stunted or deformed plants, and discoloration of coleoptile. Their results were confirmed by the writers in an examination of a large number of Manitoba farmers' samples of barley and of wheat seed from the 1937 and 1938 harvests. In these latter studies correlation coefficients of 0.893 and 0.781 were obtained between the percentage of barley and wheat seeds, respectively, found infected with *Helminthosporium sativum*, and the percentage of diseased plants in the succeeding crop.[3]

The relation of the microflora of cereal seed from the 1939 and 1940 harvests to the amount of germination and vigour of seedling growth, as well as to the prevalence of seedling blight, rootrot, and leaf-spotting diseases in the young plants was determined by means of carefully controlled greenhouse tests. The results of these tests, given in Table 4, show clearly that the amount of seedling disease in samples of wheat, oats, and barley from different crop districts in Canada in 1939 and 1940 was directly proportional to the percentage of seeds infected with pathogenic species of *Helminthosporium* and *Fusarium*. In both years, high correlation coefficients were obtained between the percentage of wheat and barley seeds infected with such species and the number of diseased seedlings arising from the seed. It is evident, therefore, that seed of small grain crops infected with *Helminthosporium* and *Fusarium* species is not good seed. Consequently, every effort should be made to produce and distribute cereal seed, particularly Foundation, Elite, Registered, and Certified seed, that is free of these disease-producing fungi.

The Prevalence and Distribution of Seed-borne Diseases of Cereals in Canada in 1939 and 1940

The relative prevalence of seed-borne diseases caused by species of *Helminthosporium* and *Fusarium* in a large number of samples of wheat, oats and barley from 8 seed inspection districts in 1939, and from 5 districts in 1940, is shown in Table 5. This table gives the average percentage of diseased samples, and the average percentage of seeds producing diseased seedlings in the infected samples from each province. No attempt is made

[3] Unpublished data, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

TABLE 4.—THE RELATION OF SEED INFECTION WITH SPECIES OF *Helminthosporium* AND *Fusarium* TO SEEDLING BLIGHT, ROOTROT, AND LEAF SPOT IN SAMPLES OF WHEAT, OATS AND BARLEY FROM THE 1939 AND 1940 CROPS, AND ACCORDING TO SEED INSPECTION DISTRICTS

Origin of seed and year produced	Number of samples examined	Percentage of seeds infected with:[1]				Percentage of seedlings diseased
		Hel.[2] *sativum*	*Hel. Avenae*	*Hel. teres*	*Fusarium* spp.	
		Wheat				
1939						
Maritime Provinces	14	6.1	0	0.1	4.3	10.6
Western Ontario	53	0.6	0	0.0	0.2	0.8
Manitoba	289	2.5	0	0.4	0.4	3.6
Saskatchewan	406	0.2	0	0.0	0.1	0.7
Alberta	223	0.1	0	0.0	0.1	1.1
British Columbia	5	0.0	0	0.0	0.1	2.9
1940						
Maritime Provinces	1	0.0	0	0.0	0.0	0.0
Quebec	3	0.7	0	0.3	2.3	0.0
Eastern Ontario	11	3.8	0	0.2	2.9	5.4
Western Ontario	16	0.4	0	0.1	0.2	0.2
Manitoba	455	2.8	0	0.3	0.9	2.5
		Oats				
1939						
Maritime Provinces	8	0.4	9.4	0.0	7.5	21.3
Quebec	6	0.1	4.5	0.0	8.9	15.5
Manitoba	16	0.7	0.8	0.1	1.0	3.6
Alberta	10	0.0	0.0	0.0	0.0	1.2
British Columbia	17	0.0	0.4	0.1	1.0	1.9
1940						
Maritime Provinces	19	0.5	7.2	0.0	6.3	7.3
Quebec	29	0.5	6.8	0.0	8.4	18.4
Eastern Ontario	100	0.2	5.6	0.1	5.1	12.2
Western Ontario	100	1.1	4.8	0.2	3.7	15.9
Manitoba	170	1.2	0.6	0.1	1.0	3.8
		Barley				
1939						
Maritime Provinces	29	35.4	0	3.6	4.4	39.7
Manitoba	141	2.7	0	0.4	0.9	4.7
Alberta	26	0.2	0	0.7	0.3	1.5
British Columbia	2	0.0	0	0.5	0.5	0.5
1940						
Maritime Provinces	4	11.7	0	0.8	0.5	3.3
Quebec	15	0.7	0	0.1	2.4	2.2
Eastern Ontario	64	4.5	0	0.2	3.4	3.4
Western Ontario	76	5.4	0	0.02	1.7	5.4
Manitoba	261	3.4	0	3.1	1.4	1.3

[1] Based on plating out 100 surface-sterilized seeds of each sample on potato dextrose agar.
[2] *Hel.* = *Helminthosporium.*

TABLE 5.—THE PREVALENCE OF SEEDLING BLIGHT AND OTHER SEED-BORNE DISEASES CAUSED BY SPECIES OF *Helminthosporium* AND *Fusarium* IN SEED SAMPLES OF WHEAT, OATS, AND BARLEY FROM THE 1939 AND 1940 CROPS, AND FROM DIFFERENT SEED INSPECTION DISTRICTS

Origin of seed and year produced	Number of samples examined	Percentage of samples diseased[1]	Percentage of plants infected[2]
		Wheat	
1939			
Maritime Provinces	14	57	18
Western Ontario	56	23	2
Manitoba	289	32	6
Saskatchewan	505	10	2
Alberta	442	12	4
British Columbia	5	0	0
All districts	1311	16.0	6.5
1940			
Maritime Provinces	1	0	0
Quebec	3	0	0
Eastern Ontario	11	27	17
Western Ontario	16	0	0
Manitoba	455	43	3
All districts	486	41.4	3.2
		Oats	
1939			
Maritime Provinces	62	90	30
Quebec	41	90	17
Manitoba	89	43	7
Alberta	150	21	4
British Columbia	27	37	7
All districts	369	46.6	19.3
1940			
Maritime Provinces	19	56	11
Quebec	29	86	21
Eastern Ontario	195	57	14
Western Ontario	237	73	17
Manitoba	170	38	8
All districts	650	59.2	15.6
		Barley	
1939			
Maritime Provinces	29	86	47
Manitoba	141	13	8
Alberta	23	13	5
British Columbia	2	0	0
All districts	195	23.6	9.4
1940			
Maritime Provinces	4	25	13
Quebec	15	13	8
Eastern Ontario	64	44	8
Western Ontario	76	45	10
Manitoba	261	10	3
All districts	420	22.5	7.3

1 Percentage of samples examined in which less than 91 per cent of the seed produced healthy seedlings.

2 Mean percentage of emerged seedlings infected in diseased seed samples.

here to discuss the prevalence of retarded, stunted, deformed, or weak seedlings in the grain samples tested, or the probable causes of these seed troubles. This is an important phase of the seed-disease problem, and the need for further investigations to determine its importance in the production of grain for seed purposes is fully realized.

From Table 5, it is evident that the average percentage of diseased samples and the percentage of infected plants from these samples were considerably lower in the Maritime Provinces in 1940 than in 1939. On the other hand, the 1940 diseased samples of oats from Quebec were just as severely infected as were those obtained in 1939 from the same province. Rather severe seedling infection occurred in the diseased samples of oats and barley from Ontario and Manitoba in 1940, the infection in those from Manitoba being just as severe as in 1939.

As might be expected from the results of the agar plate tests (Tables 2 and 3) there were striking differences each year in the prevalence of infection caused by *Helminthosporium* and *Fusarium* species in seedlings arising from samples obtained from different provinces. For example, in 1939,

TABLE 6.—THE HEALTH CONDITION OF SEED SAMPLES OF WHEAT, OATS AND BARLEY FROM THE 1939 AND 1940 CROPS, AND FROM DIFFERENT SEED INSPECTION DISTRICTS. RESULTS OF SOIL TESTS UNDER GREENHOUSE CONDITIONS

Origin of seed and year produced	No. of samples examined	Percentage of seed samples			
		Healthy		Diseased	
		High-germinating	Low-germinating[1]	High-germinating[2]	Low-germinating[3]
1939		Wheat			
Maritime Provinces	14	36	7	43	14
Western Ontario	56	64	14	22	0
Manitoba	289	60	8	30	2
Saskatchewan	505	90	2	8	0
Alberta	442	68	10	18	4
British Columbia	5	60	40	0	0
All districts	1,311	78.9	5.1	14.6	1.4
		Oats			
Maritime Provinces	62	11	0	87	2
Quebec	41	7	3	83	7
Manitoba	89	54	3	37	6
Alberta	150	73	5	17	5
British Columbia	27	56	7	26	11
All districts	369	49.6	3.8	41.5	5.1
		Barley			
Maritime Provinces	29	14	0	79	7
Manitoba	141	87	0	12	1
Alberta	23	78	9	9	4
British Columbia	2	100	0	0	0
All districts	195	75.4	1.1	21.5	2.0

TABLE 6.—THE HEALTH CONDITION OF SEED SAMPLES OF WHEAT, OATS AND BARLEY FROM THE 1939 AND 1940 CROPS, AND FROM DIFFERENT SEED INSPECTION DISTRICTS. RESULTS OF SOIL TESTS UNDER GREENHOUSE CONDITIONS—Concluded

Origin of seed and year produced	No. of samples examined	Percentage of seed samples			
		Healthy		Diseased	
		High-germinating	Low-germinating[1]	High-germinating[2]	Low-germinating[3]
1940		Wheat			
Maritime Provinces	1	0	100	0	0
Quebec	3	67	33	0	0
Eastern Ontario	11	73	0	27	0
Western Ontario	16	75	25	0	0
Manitoba	455	49	8	40	3
All districts	486	50.0	9.0	38.1	2.9
		Oats			
Maritime Provinces	19	37	5	58	0
Quebec	29	14	0	86	0
Eastern Ontario	195	40	3	46	11
Western Ontario	237	25	2	67	6
Manitoba	170	59	3	27	11
All districts	650	38.3	2.5	50.9	8.3
		Barley			
Maritime Provinces	4	75	0	25	0
Quebec	15	86	0	7	7
Eastern Ontario	64	50	6	39	5
Western Ontario	76	50	5	40	5
Manitoba	261	86	4	9	1
All districts	420	73.6	4.5	19.3	2.6

1 Germination not improved by seed treatment. Increased rate of seeding recommended to grower.
2 Samples of high germinability, but requiring seed treatment for the control of seedling blight and leaf-spotting diseases.
3 Germination not improved by seed treatment. Seed treatment and increase in rate of seeding recommended to grower.

infection in diseased samples was most severe in those from the Maritime Provinces, while infection was relatively light in those from Saskatchewan, Alberta, and British Columbia. In 1940, the percentage of infection in samples of oats and barley from the Maritime Provinces was high, and although lower than in 1939, was considerably higher than in the 1940 diseased samples of oats and barley from Manitoba. A large proportion of the oat samples obtained from Quebec in both 1939 and 1940 was diseased, and the degree of seedling infection was relatively severe in both years.

To show more clearly the relative prevalence and distribution of seed-borne diseases of cereals in Canada in 1939 and 1940, the results of the greenhouse soil tests are summarized in Table 6. In this table, the healthy and diseased samples have been separated into high-germinating and low-germinating samples. A full description of these sample classes is given in Tables 6 and 8.

The results in Table 6 show that, in 1939, relatively disease-free seed of wheat, oats, and barley was produced in the Prairie Provinces and in British Columbia, while severely infected seed, particularly that of oats and barley, was produced in the Maritime Provinces and Quebec. Again, in 1940, the most severely infected samples of oats and barley were obtained from Quebec and the Maritime Provinces, respectively, while the cleanest oats and barley seed examined was obtained from Manitoba.

TABLE 7.—THE PREVALENCE OF SURFACE-BORNE SMUTS IN SEED SAMPLES OF WHEAT, OATS, AND BARLEY FROM THE 1940 CROP, AND FROM DIFFERENT SEED INSPECTION DISTRICTS

Origin of seed[1]	Wheat		Oats		Barley	
	No. of samples examined	Percentage of samples with smut[2]	No. of samples examined	Percentage of samples with smut[2]	No. of samples examined	Percentage of samples with smut[2]
Maritime Provinces	1	0	19	5	4	25
Quebec	3	0	29	3	15	27
Eastern Ontario	11	0	195	12	64	16
Western Ontario	16	12	237	12	76	13
Manitoba (P.P.D.)	65	0	43	12	43	37
Manitoba (M.F.)	390	0.7	127	53	218	78
All samples	486	0.8	650	15.5	420	50.0

[1] P.P.D. = Manitoba Plant Products Division samples.
M.F. = Manitoba Farmers' samples.
[2] Samples with a smut spore load exceeding 1 : 128,000.

The prevalence of surface-borne smut in seed samples of wheat, oats and barley from the 1940 harvest, as based on a maximum allowable spore load of 1 : 128,000 is shown in Table 7[3]. As the data in this table show, the great majority of the 1940 wheat samples tested were virtually free of smut. On the other hand, it was found that 12% of the oat samples from Ontario and Manitoba, submitted by the Plant Products Division, carried a smut spore load sufficiently heavy to justify seed treatment. Of the 127 seed samples of oats examined from Manitoba farms, 53% required treatment for smut. An appreciable proportion of the 1940 barley samples submitted by the Plant Products Division from Eastern Canada and Manitoba were found to be carrying a fairly heavy load of smut spores, and hence required seed treatment. As might be expected, the percentage of smutty samples in the ordinary Manitoba farmers' seed stocks of barley was much higher than in the barley samples of Elite, Registered, and Certified seed submitted by the Plant Products Division from Manitoba. As the data in Table 7 show, 78% of the 218 Manitoba farm samples of barley carried what is at present considered a dangerous load of smut spores.

The Relation of Seed-borne Diseases of Wheat, Oats, and Barley to Germination

The results of the soil germination tests for samples of wheat, oats, and barley obtained from the 1939 and 1940 crops are summarized in Table 8. The data in this table show that the average germination for the

[3] The writers are indebted to Mr. W. J. Cherewick, Dominion Laboratory of Plant Pathology, Winnipeg Man., for carrying out the smut tests reported in this paper.

healthy samples was, in both years, about 95%. Thus, the healthy seed was of high vitality. The average percentage of germination in the diseased seed samples was from 4 to 7% less than that in the healthy samples. This indicates that the presence, in or on the seed, of disease-producing organisms, does not in general affect appreciably the germination of the seed, and thus confirms the results of earlier unpublished studies in which it was found that differences in germination between healthy seed and diseased seed is usually so small that this criterion alone cannot be used as a reliable index of the value of a grain sample for seed. Naturally, the general development of the seedlings also must be taken into consideration, but the most important criterion for determining the pathological condition of cereal seed samples is the amount and degree of disease infection occurring on young plants arising from the seed.

TABLE 8.—THE RELATION OF SEED-BORNE DISEASES CAUSED BY SPECIES OF *Helminthosporium* AND *Fusarium* TO GERMINATION IN UNTREATED SEED SAMPLES OF WHEAT, OATS, AND BARLEY FROM THE 1939 AND 1940 CROPS. (DATA ARE MEANS OF ALL SAMPLES EXAMINED IN 1939 AND 1940)

Crop and year seed produced	Percentage Germination in Soil			
	Healthy samples[1]		Diseased samples[2]	
	Number examined	Percentage germination	Number examined	Percentage germination
Wheat				
1939	1101	95.1	210	90.5
1940	287	95.1	199	88.0
Oats				
1939	197	96.5	172	91.3
1940	265	95.4	385	91.3
Barley				
1939	149	97.3	46	91.1
1940	328	97.1	92	91.7

[1] Seed samples in which at least 91 per cent of the seeds produced healthy seedlings.
[2] Seed samples in which less then 91 per cent of the seeds produced healthy seedlings.

THE EFFECT OF SEED TREATMENT ON GERMINATION AND ON THE CONTROL OF SEED-BORNE DISEASES IN CEREALS

The pathological analysis of cereal seed samples from the crops of 1939 and 1940 provided an excellent opportunity to study the effect of seed treatment on germination, plant growth, and on the development of seedling diseases. As previously described, treated and untreated lots of each seed sample were sown in beds of ordinary soil in the greenhouse. About 10 days after planting, the seedlings arising from the seed were examined for seed-borne diseases. The results of the effect of seed treatment on germination, and on the development of seed-borne diseases in wheat, oats, and barley, are given in Tables 9, 10 and 11.

The average results for all districts in Tables 9 and 10 show that seed treatment with an organic mercury dust had no appreciable effect on the germination of healthy seed of wheat, oats, and barley, i.e., uninjured seed that was virtually free of pathogenic fungi. In 1939, for instance, the

TABLE 9.—THE EFFECT OF SEED TREATMENT ON THE GERMINATION OF HEALTHY AND DISEASED SEED SAMPLES OF WHEAT, OATS, AND BARLEY FROM THE 1939 AND 1940 CROPS. (DATA ARE MEANS OF ALL SAMPLES EXAMINED IN 1939 AND 1940)

Percentage Germination in Soil

Crop and year seed produced	Healthy samples[1]			Diseased samples[2]		
	Number examined	Natural seed	Treated seed[3]	Number examined	Natural seed	Treated seed[3]
Wheat						
1939	1101	95.1	95.9	210	90.5	95.2
1940	287	95.1	95.5	199	88.0	96.0
Oats						
1939	197	95.0	96.5	172	91.3	95.7
1940	265	95.4	94.3	385	91.3	94.6
Barley						
1939	149	97.3	97.7	46	91.1	95.7
1940	328	97.1	96.7	92	91.7	95.1

[1] and [2]. See Table 8.
[2] Seed treated with an organic mercury dust.

average germination for the healthy, untreated seed of 1,101 samples of wheat was 95.1%, while the average germination of treated seed lots from the same 1,101 samples was only 95.9%. Seed treatment, on the other hand, materially improved the germination of the infected or damaged cereal seed samples, the average increase in germination in all crops and for all districts being approximately 5%. The results in Tables 9 and 10 show that the average percentage germination of the treated, diseased seed samples was always almost identical with that of the healthy, untreated samples. It was found that most of the seed samples that were not improved by seed treatment were virtually free of pathogenic fungi, although

TABLE 10.—THE EFFECT OF SEED TREATMENT ON THE GERMINATION OF NATURALLY LOW-GERMINATING SEED SAMPLES OF WHEAT, OATS, AND BARLEY FROM THE 1939 AND 1940 CROPS. (DATA ARE MEANS OF SAMPLES EXAMINED IN 1939 AND 1940)

Percentage Germination in Soil

Crop and year seed produced	Healthy samples[1]			Diseased samples[2]		
	Number examined	Natural seed	Treated seed[3]	Number examined	Natural seed	Treated seed[3]
Wheat						
1939	67	88.3	89.0	19	83.1	87.8
1940	44	89.8	89.9	14	80.0	89.0
Oats						
1939	28	85.5	84.0	38	74.8	83.2
1940	15	84.5	82.3	44	77.4	83.0
Barley						
1940	19	83.6	82.8	10	73.5	76.6

[1] and [2]. See Table 6.
[2] Seed treated with an organic mercury dust.

many were heavily infected with other fungi, especially with species of *Alternaria*. The results show clearly that, in so far as germination is concerned, there is little or no value in treating clean seed of wheat, oats, and barley, if it is sown in clean soil. This conclusion agrees with that reached by Leukel and Stanton (9) with oats, and by Kiesselbach (7, 8) with corn. Furthermore, the results of the greenhouse tests herein reported are in accordance with the results of extensive seed treatment field tests with wheat recently reported by the writers (11).

A certain number of healthy and diseased samples of wheat, oats, and barley from the harvests of 1939 and 1940 were of low vitality, that is to say, less than 91% of the seeds germinated when sown in soil beds in the greenhouse. The germination results of treated and untreated lots of this particular group of samples are summarized in Table 10. It is evident from this table that, in healthy seed of this type, germination was not improved appreciably by treatment with an organic mercury dust. As might be expected, however, the germination in the diseased samples of this group was materially improved by seed treatment, but in no case was the germination higher than in the untreated, healthy, naturally low-germinating samples. In other words, the improvement in germination brought about by seed treatment of the low-germinating diseased samples was

TABLE 11.—THE EFFECT OF SEED TREATMENT ON THE CONTROL OF SEEDLING BLIGHT, AND ROOT-ROTTING AND LEAF-SPOTTING DISEASES CAUSED BY SPECIES OF *Helminthosporium* AND *Fusarium* IN SAMPLES OF WHEAT, OATS, AND BARLEY FROM THE 1939 AND 1940 CROPS

Percentage of Plants Diseased[1]
(Data are means of district samples)

Origin of seed and year produced	Wheat			Oats			Barley		
	No. of samples examined	Natural seed[2]	Treated seed[3]	No. of samples examined	Natural seed	Treated seed	No. of samples examined	Natural seed	Treated seed
1939									
Maritime Provinces	8	17.6	0.9	55	30.0	0.1	25	46.6	1.2
Quebec	0	—	—	37	16.7	0.0	0	—	—
Western Ontario	13	1.9	0.2	0	—	—	0	—	-
Manitoba	94	6.4	0.6	38	6.6	0.2	18	8.3	0.6
Saskatchewan	43	2.0	0.2	0	—	—	0	—	—
Alberta	52	4.4	0.1	32	3.9	0.2	3	4.9	0.2
British Columbia	0	—	—	10	6.8	0.3	0	—	—
Average	(210)	6.5	0.4	(172)	19.3	0.1	(46)	9.4	0.9
1940									
Maritime Provinces	0	—	—	11	10.6	0.1	1	13.0	0.0
Quebec	0	—	—	25	21.0	0.0	2	7.5	0.0
Eastern Ontario	3	17.2	0.0	111	14.1	0.1	28	8.1	0.1
Western Ontario	0	—	—	173	17.1	0.1	34	10.2	0.1
Manitoba	196	3.0	0.4	65	7.9	0.1	27	3.0	0.1
Average	(199)	3.2	0.4	(385)	15.6	0.1	(92)	7.3	0.1

[1] Percentage of emerged seedlings with disease lesions on roots, stems, or leaves. 0.1 per cent represents a very light trace of infection; that is, only an occasional diseased seedling.
[2] Natural, untreated seed.
[3] Seed treated with an organic mercury dust.

entirely due to the control of seed-borne diseases, and not to any stimulatory action of the treatment. In reporting the results of the low-germinating cereal samples to growers, an increase in seeding rate was always recommended.

The results of seed treatment on the control of seed-borne diseases of cereals caused by *Helminthosporium* and *Fusarium* species are shown in Table 11. It is evident that treatment with an organic mercury dust decreased the number of plants that were attacked or destroyed by these diseases, the decrease being proportionate to the amount of infection. In other words, the higher the percentage of seed infected, the more beneficial was the treatment. Almost complete control of seedling blight, rootrot, and leaf spot of wheat, oats, and barley caused by the above-mentioned species of fungi was obtained by treating the seed with an organic mercury dust, and similar results were obtained in the control of leaf blotch of oats caused by *Helminthosporium Avenae*, and of net blotch of barley caused by *H. teres*.

DISCUSSION

The results of the present seed-borne disease studies have shown that many different kinds of organisms are carried by cereal seed. Of these, the fungi are the most important and destructive ones. Cereal seeds severely infected with fungi are often discoloured, and the occurrence of discoloured kernels in the threshed grain lowers the market value of the crop. A typical example of this type of seed discoloration in wheat is known as kernel smudge, or black-point, which, in some years, causes appreciable losses in certain grain-growing districts. owing to lowering of the commercial grade (10). From the present work it is evident that, in addition to causing losses in commercial grain quality, certain fungi found in cereal seeds are pathogenic, and when seed carrying these organisms is sown, a greater or less number of the resulting seedlings are attacked and destroyed.

The 1940 seed-borne disease survey indicates that a large proportion of the seed samples of oats and barley examined were heavily contaminated with smut spores. As the surface-borne smuts of cereals are largely preventable by seed treatment, the fact that they are still very prevalent indicates that seed treatment is not being adequately practised for these coarse grain crops. As a matter of fact, it is evident from the present studies that, although practical and adequate methods of seed treatment are available, they are not widely enough employed in this country against the surface-borne smuts, seedling blight, rootrot, and leaf-spotting diseases of wheat, oats, and barley.

The results of the present investigation indicate that seed-borne diseases are very prevalent in certain districts of Canada, while other areas are likely to produce seed that is free, or relatively free, of fungi that cause seedling blight, and other destructive diseases. Consequently, it is important that all practicable means be employed in this country to prevent the spread of plant pathogens by seed. Perhaps one of the most effective means of doing this is to determine the health condition of the seed before is is used for seed purposes. In recent years, seed analysts and others concerned with good seed production have manifested considerable interest in the phytopathological aspects of seed testing, and it would seem that

the time is not far distant when the pathological condition of the seed will be determined in the ordinary course of seed analysis. In Holland, chiefly through the efforts of Doyer (4, 5), a pathological examination of seed samples has become a regular part of the seed analysts' work. In this important work the plant pathologist and seed analyst have a very direct responsibility: the former to outline methods of detection, identification and control of disease; the latter to utilize these methods in the best interests of the seed grower, seed tradesman, and buyer of seed. It is certain that a pathological seed service would render a valuable service to the grain-growers of Canada.

From the results given in this paper, the practical value of annual seed-borne disease surveys and pathological seed tests is obvious. They provide information that is of practical value to the grain-grower. Each year, he can be informed regarding the condition of his seed stock. If it is diseased, mechanically injured, or damaged in any other way, he can be warned against its use for seed purposes, and can be advised whether or not seed treatment or increase in rate of seeding will be beneficial. Such information would help to protect the grower from crop losses, and help to prevent the inter-regional distribution of pathogenic seed-borne organisms in Canada.

Although the present study has been largely of a preliminary nature, it has, nevertheless, furnished valuable information concerning the relative prevalence and distribution of certain important seed-borne diseases of small grain crops. Furthermore, it has emphasized the need for additional information regarding certain aspects of the problem, including the effect of climate on disease development, and the relative resistance of cereal varieties to seed-borne diseases caused by species of *Helminthosporium* and *Fusarium*. It is therefore important that research work on a comprehensive basis should be consistently pursued. Naturally, a detailed study of all aspects of the general seed-borne disease problem is desirable, but the development of suitable techniques for detecting seed-borne diseases in seed laboratory practice, the development of effective control measures, and the establishment of limits of tolerance for the most important seed-borne diseases, are some of the more immediate and concrete problems for the plant pathologist. No doubt, an early solution of some of these problems will result from the active research on seed-borne diseases now being carried out in various plant pathological laboratories of the Dominion Department of Agriculture throughout Canada.

SUMMARY

Seed surveys and pathological tests were made to determine the relative prevalence of disease-producing organisms in a large number of samples of wheat, oats, barley, and rye seed of the 1939 and 1940 crops from the different seed inspection districts in Canada, and to ascertain the effect of seed-borne parasites on the succeeding crop.

Many species of fungi and bacteria were isolated from the samples. In all of the samples, species of *Alternaria*, were by far the most common fungi, but cultural, microscopical, and pathological tests showed that,

apart from the smut fungi, species of *Helminthosporium* and *Fusarium* were the most important disease-producing organisms. Species of *Helminthosporium* were much more prevalent that those of *Fusarium* in wheat and barley seed, but species of *Fusarium* were isolated just as frequently from oat seed as was *Helminthosporium Avenae*, the predominating species of *Helminthosporium* carried by this seed.

Seedling blight, rootrot, and certain destructive leaf-spotting diseases of wheat, oats, and barley, developed from seeds infected with *Helminthosporium sativum*, *H. Avenae*, and *H. teres*, respectively. High positive correlations were found between the percentage of seeds infected with *H. sativum* and *H. Avenae* and the amount of disease present in the resulting seedlings.

In 1939, samples of oats and barley seed obtained from Manitoba, Alberta, and British Columbia were relatively free from infection by *Helminthosporium* and *Fusarium* species, while those from the Maritime Provinces, Quebec, and Ontario were severely infected with these fungi. The cleanest wheat seed examined in 1939 was from Saskatchewan. In 1940, seed samples of oats and barley from the Maritime Provinces were more severely infected with *Helminthosporium* and *Fusarium* species than were those from Manitoba. The surveys thus indicate that certain areas in Canada are much more likely than others to produce seed free, or relatively free, from such organisms.

An examination of the seed samples from the 1940 crop indicates that, in all districts surveyed, wheat seed was comparatively free of smut spores, but a large percentage of the seed samples of oats and barley was carrying a sufficiently heavy spore load of smut to require seed treatment. This result suggests that more attention should be given in Canada to the treatment of oats and barley for the control of smut.

Treating seed of wheat, oats and barley infected with species of *Helminthosporium* and *Fusarium* with a mercurial dust improved seed germination, and gave almost complete control of seedling blight, seedling rootrot, leaf spot, and leaf blotch.

Extensive soil tests showed that the germination of clean, uninjured seed was not improved by seed treatment, and that there is little or no value in treating clean, uninjured seed of wheat, oats, and barley, if sown in clean soil.

Lightly infected wheat, oats, and barley seed treated with an organic mercury dust was just as good for seed purposes as was healthy seed.

The results of the present investigation indicate that annual seed-borne disease surveys are capable of rendering a valuable service to seed producers and grain growers.

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STUDIES ON SURFACE TAINT BUTTER

III. SOME FURTHER CHARACTERISTICS OF *PSEUDOMONAS PUTREFACIENS*[1]

H. Wolochow[2], H. R. Thornton[3] and E. G. Hood[4]

[Received for publication October 17, 1941]

The present study is an enlargement of the description of *Pseudomonas putrefaciens* given by Derby and Hammer (3). The taxonomic terms, *Pseudomonas putrefaciens* and *Achromobacter putrefaciens*, are used synonomously (14).

METHODS

Unless otherwise stated, the procedures outlined in Manual of Methods for Pure Culture Study of Bacteria (S.A.B.) were followed. When temperatures are not specified, incubation was at the temperature of the room, about 21° C. Except as otherwise stated, the same strain of the organism as previously reported (11, 12) was used and was found to conform essentially to Derby and Hammer's description.

Temperature

This strain of the organism grew on nutrient agar slants at 2° C. and 32° C. but not at 37° C.

The results of the Iowa workers (3, 1, 8), which show that the organism is killed during pasteurization, were confirmed. One drop of a water suspension of the organism from 2- to 3-day agar slant cultures was mixed with 5 ml. of sterile skim milk held in a bath at the required temperature. Two drops of this skim milk suspension were transferred at the end of the specified time to 9 ml. of nutrient broth which was then incubated for 5 days at 21° C. As measured by growth in the incubated broths, the organism survived 54.4° C. (130° F.) for 5 minutes but did not survive the following temperature-time combinations:

54.4° C. (130° F.) for 10 minutes
57.2° C. (135° F.) for 5 minutes
60.0° C. (140° F.) for 5 minutes
62.8° C. (145° F.) for 1 minute
65.6° C. (150° F.) for ½ minute

Pleomorphism and Cultural Phases

Pleomorphic forms were noticed when *Ps. putrefaciens* was grown in 1% LiCl broth, 6% NaCl broth, 4% NaCl in skim milk, and were also observed in various synthetic media and in old broth and skim milk cultures.

On nutrient agar the typical surface colony was radially striated with indented, smooth edges, while the individual cells were regularly rod-shaped (Plate I, 1 and 4). On nutrient agar containing 5% gelatin the surface colonies were circular with smooth surfaces and edges (Plate I, 7).

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[1] Joint contribution from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, and the Department of Dairying, University of Alberta, Edmonton. Contribution No. 133 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

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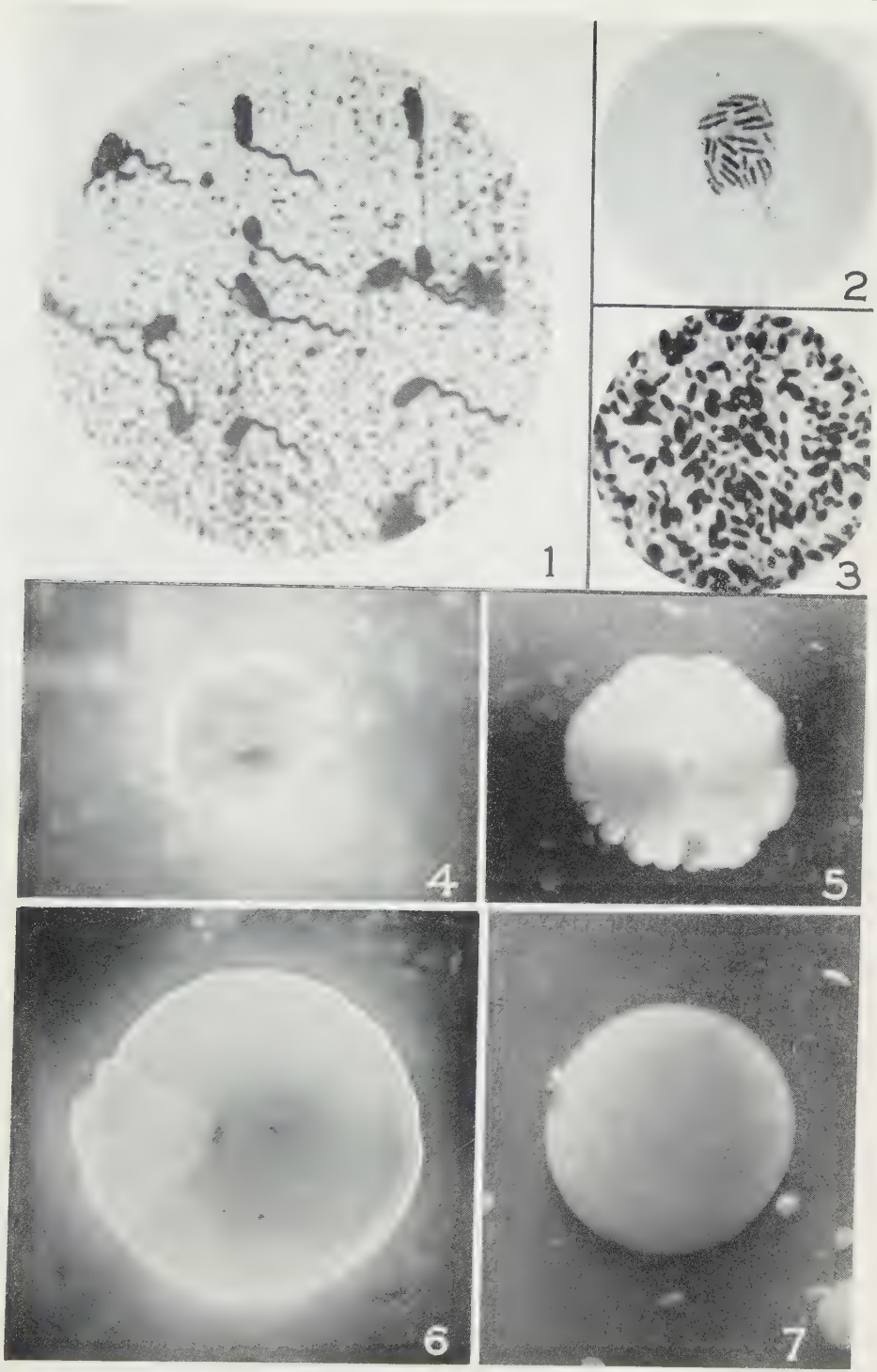

PLATE 1. 1. *Ps. putrefaciens* from nutrient agar slant culture showing flagellation. 2. Stained smear from rough segment of colony shown in 6. 3. Stained smear from smooth portion of colony shown in 6. 4. Colony of *Ps. putrefaciens on* nutrient agar. 5. Colony of *Ps. putrefaciens* on nutrient agar containing 1% gelatin. Smooth colonies as shown in 7 also appeared on this medium. 6. A colony of *Ps. putrefaciens* on nutrient agar containing 1% gelatin showing two colonial types in the same colony. 7. Colony of *Ps. putrefaciens* on nutrient agar containing 5%

Smears from this colonial type exhibited pleomorphic forms. On nutrient agar containing 1% gelatin both types of colonies were to be found (Plate I, 5) and sometimes a segment of the first colonial type was observed within a colony of the second type (Plate I, 2, 3 and 6).

The implications of these findings on the practical problems of surface taint would seem to deserve further investigation. This is particularly true in view of the ease with which pleomorphism may be induced by NaCl.

Bacteriostasis and Gram Characteristics

At no time was it possible to demonstrate a gram reaction other than negative. In glucose nutrient agar, growth was inhibited by crystal violet in concentrations greater than 1:40,000 and by brilliant green in a concentration of 1:200,000. Higher dilutions of brilliant green were not tried. In tryptone, water growth was inhibited by mercurochrome in a concentration between 1:2,500 and 1:5,000; and in tryptone-glucose-beef-extract-2% skim milk agar, growth was inhibited in a concentration of 1:10,000. These results indicate the strongly gram-negative characteristics of the organism.

Photic Characteristics

Colonies or streaks of this organism growing on any of the solid media which were tried had a brownish-white colour by reflected light, and a transparent, brownish-red colour by transmitted light and showed marked iridescence at the edges. Chromogenesis was not observed in liquid media, in the glycerol or ethanol medium of Turfitt (9) or the asparagin medium of Georgia and Poe (4).

Growth in Various Media

Growth was much better on peptone-beef-extract agar, peptone-veal-infusion agar, or tryptone-glucose-beef-extract-2% skim milk agar than on peptone agar alone or beef-extract agar alone. The organism grew better in nutrient broth than in skim milk. An alkaline reaction was observable around the colonies growing on Simmon's citrate agar. A slight tendency to confluence was the only distinguishing feature noticed about the colonies growing on Levine's eosine-methylene blue agar. Growth was slow on violet red bile agar, no precipitation of the bile being evident. The reddish colour of the medium immediately around each surface colony was discharged, the halo becoming wider with age.

The organism did not grow in the medium of Gray and Thornton (5) when phenol or meta-cresol was the sole source of carbon. Growth occurred in nutrient broth containing 0.1% of Duponol W. A. powder but there was no growth in the presence of 1% of the powder.

Lipolysis

It was not possible to demonstrate lipolysis of butterfat by *Ps. putrefaciens* as measured by the nile blue sulphate and natural fat methods of Long and Hammer (7).

pH

Growth initiation, as measured by microscopic examination after 2 days at room temperature, in tubes of broth containing McIlvaine's buffers was found to be as set forth in Table 1. It is seen that growth did not take place with certainty at a pH lower than 5.48 and was enhanced as the pH was raised. In plain broth adjusted with sterile lactic acid the lower limit of growth was pH 5.6.

TABLE 1.—GROWTH IN BUFFERED BROTH

Initial pH	pH after 2 days		Growth
	Control	Inoculated	
5.14	5.08	5.08	−
5.26	5.27	5.26	?
5.34	5.27	5.27	?
5.48	5.48	5.48	+
5.76	5.67	5.67	+
6.00	5.99	5.99	++
6.25	6.20	6.20	++
6.50	6.51	6.51	+++

− No growth.
+ Growth.
? Questionable growth.

TABLE 2.—GROWTH IN BUFFERED PEPTONE WATER

Initial pH	At 2 days		At 6 days	at 9 days	
	pH	Growth	Growth	pH	Growth
7.5	7.75	++	+++	7.48	+++
8.16	8.15	++	+++	8.75	+++
8.20	8.18	−	−	8.10	−
8.72		−	−		−
9.10		−	−		−
9.32		−	−		−

In 0.5% peptone water buffered at various pH levels by means of McIlvaine's buffers the upper pH limit of growth initiation was found to be 8.15 (Table 2). In skim milk adjusted by varying additions of sterile NaOH the upper pH limit of growth initiation was found to lie between 9.5 and 10.72 (Table 3).

TABLE 3.—GROWTH IN ADJUSTED SKIM MILK

Initial pH	At 2 days		At 6 days
	pH	Growth	Growth
6.72		++	++
7.30	7.09	++	++
7.70	7.30	++	++
8.25	7.62	++	++
8.50	7.64	++	++
9.00	8.25	++	++
9.50	8.54	+	++
10.72	9.50	−	−

Since there appeared to be a tendency for the organism to lower the higher pH's of skim milk, the pH changes in sterile skim milks of varying initial pH levels (adjustment by means of NaOH and H_2SO_4) were followed for 6 days before inoculation. At the end of 6 days the milks were inoculated with *Ps. putrefaciens* and the pH changes followed for another 3 days. From Figure 1 it is seen that the organism brought the pH's toward a common centre, around pH 6.5.

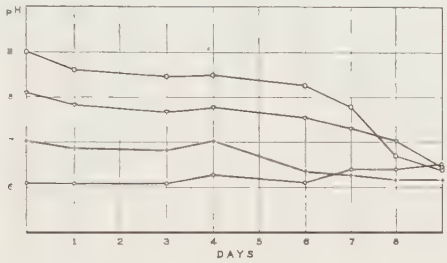

FIGURE 1. pH changes in adjusted skim milks inoculated on the sixth day.

In a repetition of this experiment the pH changes were followed in uninoculated controls and concurrently in their duplicates inoculated immediately after preparation. From Figure 2 it is seen that there was a less marked tendency for the acidities to drift toward a common centre. In both experiments there was also a tendency for the sterile controls to drift toward a central level.

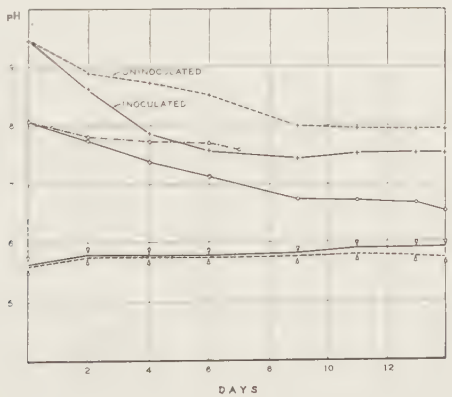

FIGURE 2. pH changes in adjusted skim milks inoculated immediately.

These findings were presented by Wolochow (10) in preliminary form and have been generally confirmed (8). It is evident that *Ps. putrefaciens* is able to initiate growth and to grow over a pH range considerably wider than is commonly found in the serum of normal or surface taint Alberta butters.

E_h

Derby and Hammer (3) reported rapid reduction of litmus in milk by *Ps. putrefaciens* and the present authors consider the organism to be

strongly reducing. Since, as was shown in the first paper of this series, there is a relation between the E_h of the milk and odour production by the organism, E_h changes in different media were followed with the results shown in Figure 3.

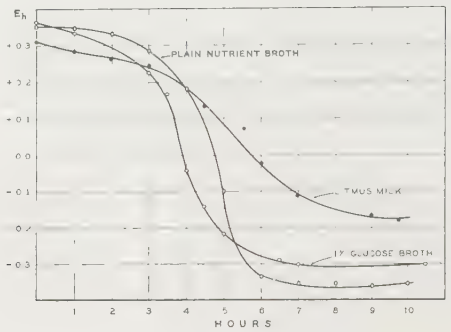

FIGURE 3. E_h-time curves in three media.

These E_h-time curves provide a seemingly plausible explanation for the observation that nutrient broth is a better medium than skim milk for the growth of this organism, because the former is less strongly poised than the latter. On the other hand Claydon and Hammer (1) were unable to obtain more consistent plating results when the E_h of the medium was altered by the addition of various substances. Since they do not report their E_h values, it is difficult to evaluate their results. Further study of this problem seems warranted.

NaCl

Motility was not observed in cultures of *Ps. putrefaciens* grown in nutrient broth or skim milk containing 2% NaCl. Growth occurred in 3 days in nutrient broth containing 6% but not 8% NaCl. Growth occurred in 3 days in skim milk in the presence of 4% but not 6% NaCl. Growth was demonstrated in extracted butter serum of 1.2% but not 6.8% NaCl content.

Nitrogen

Nitrogen distribution in skim milk cultures of *Ps. putrefaciens* was followed for 19 days. The results are presented in Figure 4. The non-protein nitrogen (NPN) was determined by the Kjeldahl method applied to the fraction not precipitated by trichloracetic acid. Formol nitrogen, which includes ammonia and free amino nitrogen, was measured by the formol titration method, and ammonia was determined by titration of the distillate from the alkalinized culture medium.

A glance at Figure 4 suffices to show that this organism is indeed proteolytic in milk and provides a possible explanation for the production of the sweaty feet substance as well as fatty acids. It may be that the reductive deamination of the freed amino acids liberates acidic compounds which are soluble in fat solvents. The sweaty feet odour was not noticed until after the NPN curve began to rise steeply. It is possible that these may be unrelated simultaneous occurrences but the possibility that the odour compound arises from protein decomposition is attractive.

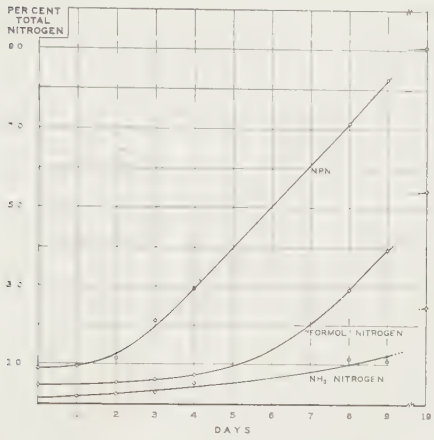

FIGURE 4. Nitrogen distribution in skim milk cultures.

The availability of nitrogen in 9 nitrogen-containing compounds was studied by adding the compound to be tested to Kisch's solution which was made up of CP chemicals. Glucose was added to part of this medium to form a 1% solution while in the other part of the medium sucrose replaced the glucose. Tubes of these different media were inoculated with *Ps. putrefaciens* and incubated up to 13 days. Growth was determined on the fourth transfer by turbidity and microscopic examination. The results are to be found in Table 4. Meagre growth was also demonstrated in a

TABLE 4.—NITROGEN AVAILABILITY IN MODIFIED KISCH'S SOLUTION

Added substance	Percentage conc.	Growth	
		Glucose	Sucrose
No addition	0	−	−
Asparagin	0.6	+	+
Creatine	1	+	+
Casein (Hammersten)	4	+	+
Sodium caseinate	2	+	+
Urea	4	−	+
$(NH_4)_2\ SO_4$	0.4	−	+
$NH_4\ H_2\ PO_4$	0.4	+	+
KNO_2	0.8	−	−
KNO_3	0.8	−	+

phosphate-buffered asparagin-water inoculated by needle from a slant culture. The culture has been carried through 5 generations on Koser's citrate medium in which the only nitrogen source is sodium ammonium phosphate. On the other hand the organism did not attack protamines (methyl or ethyl amine) in the presence or absence of glucose when the technique of den Dooren de Jong (2) was used. Nevertheless, these results leave no room for doubt that this organism is able to metabolize simple organic and inorganic forms of nitrogen.

Sulphur

This strain of *Ps. putrefaciens* gave no evidence of H_2S production in lead acetate agar (Difco). As measured by the lead acetate paper test, H_2S was not produced in the following media:—

1. Skim milk powder up to 10% in water.
2. Nutrient broth.
3. Nutrient broth plus 0.1% methionine.
4. Nutrient broth plus less than 1% or more than 5% skim milk powder.
5. Reconstituted skim milk powder 1 :10 in water plus 0.0066%, or less, cystine.

On the other hand H_2S was produced in the following media:—

1. Nutrient broth plus from 1% to 5% skim milk powder.
2. Nutrient broth plus from 1% to 10% skim milk powder and 0.012% cystine, although H_2S production was delayed in the presence of 5% to 10% skim milk powder.
3. Skim milk powder reconstituted 1 :10 in water plus 0.012% cystine.
4. Skim milk powder reconstituted 1 :10 in water plus 0.003 p.p.m. to 27.9 p.p.m. $FeSO_4$.
5. Skim milk powder reconstituted 1 :10 in 0.005 N $Na_2S_2O_3$.
6. Rennet whey neutralized to pH 7 with sterile NaOH after autoclaving.
7. Autoclaved aqueous egg albumen.

A possible interpretation of these observations is that there are concurrently in milk an available H_2S source, presumably the sulphydril compounds, and an H_2S-suppressing factor. Further investigation, however, is necessary to justify any conclusion in this regard.

It is interesting to note that the production of the sweaty feet odour took place only in the presence of milk solids and that there was a tendency for the sweaty feet odour and H_2S not to be produced simultaneously. In those borderline cases in which both were produced, one or the other was present in sufficiently low concentration to make positive identification somewhat questionable. Since there is no sulphur in the steam-distillate residue containing the sweaty feet odourous compound, it would be indiscreet at present to comment further on any possible relation between odour production and sulphur metabolism.

Carbohydrates

The pH of lactose broth cultures of this strain was consistently brought to, and remained on, the alkaline side of neutrality. An acid condition was consistently observed in sucrose broth cultures with pH's as low as 6.1. In maltose broths the pH's gradually fluctuated, usually to the acid side, and on one occasion a pH value below 6.0 was observed. The hydrogen ion concentrations in glucose broths fluctuated in general between pH 6.9

and 7.2, but were as low as pH 6.5. In Figure 5 the curves for nutrient, lactose, and sucrose broths are representative of a number of trials in which the initial acidity was on the acid side of neutrality. The maltose and glucose curves indicate greater consistency than was observed in certain other trials, although in all cases the general trend of the curves was somewhat similar. The colour changes of brom thymol blue in carbohydrate broth cultures were in harmony with these results. These results were presented in preliminary form by Wolochow (10) and have since had general confirmation (1, 6, 8). The results of reducing-sugar determinations in glucose broth cultures were inconsistent and not reproducible. This was, presumably, because of the interference of other reducing substances produced by the organism.

FIGURE 5. pH changes in carbohydrate broths.

Copper-reducing substances, as shown by Benedict's reagent, were produced when the organism was grown for 4 to 30 days in nutrient broth containing 0.2% starch. The typical purple colour of the starch-iodine reaction was obtained in 4-day cultures but in 14-day cultures a brown colour was observed. The pH of the cultures was not lowered during this time.

No copper-reducing substances, as shown by Benedict's reagent, were produced in nutrient broth containing filter paper strips.

Volatile Acids

It has been shown (12) that *Ps. putrefaciens* produces acetic, butyric and isovaleric acids as measured by the Duclaux constants of steam-distillate residues from skim milk cultures. When the skim milk cultures were shaken once per day during incubation, the same procedures recovered as high as three times as much volatile acid as when the cultures were incubated undisturbed.

OTHER ISOLATIONS OF *PS. PUTREFACIENS*

By the criteria of morphology, gram reaction and typical behaviour in litmus milk, 43 isolations from water and butter (14) were judged to be *Ps. putrefaciens*. Three of these cultures were lost. The remaining 40 cultures conformed to the classical description of this organism except for the following characteristics.

Motility was not observed in a few strains and there was a slight variation in the shape and colour of nutrient agar colonies in a very few strains. Thirty-one of the 40 strains did not grow on agar slants at 37° C., 15 strains did not grow at 32° C., and 2 strains did not grow at 2° C. Nitrite production was not demonstrated in 7 cultures, while H_2S production in nutrient broth was quite variable. One strain did not liquefy gelatin, and another strain, which gave a positive Voges-Proskauer test, produced a very mild sweaty feet odour in milk and did not cause surface taint in butter. Three cultures did not produce surface taint in experimental butter. As measured by the colour changes of brom thymol blue in 1% carbohydrate broths, the fermentation of glucose, sucrose, lactose and maltose was so variable as to render this procedure useless as a differential technique (Table 5).

TABLE 5.—THE NUMBER OF CULTURES OF *PS. PUTREFACIENS* PRODUCING ACIDITY IN CARBOHYDRATE BROTHS

Sugar	Acid reaction	Non-acid reaction
Glucose	20	20
Sucrose	21	19
Lactose	11	29
Maltose	25	15

The variations in the characteristics of these strains of *Ps. putrefaciens* are, in general, similar to those reported by Long and Hammer (8). In certain details the results of the two investigations may seem to be incompatible. This, however, is to be expected in view of differing methods of isolation and recognition and in view of the ease with which dissociation of this organism may be induced.

SUMMARY

Some further characteristics of 41 strains of *Ps. putrefaciens* are described and discussed.

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11. I. Odour production by *Pseudomonas putrefaciens*. Sci. Ag. 22 : 277-286. 1942.

12. II. An odourous compound in skim milk cultures of *Pseudomonas putrefaciens*. Sci. Ag. 22 : 347-355. 1942.

13. III. Some further characteristics of *Pseudomonas putrefaciens*.

14. IV. Distribution and taxonomy of *Pseudomonas putrefaciens*. To be published.

15. V. The growth of *Pseudomonas putrefaciens* in butter. To be published.

16. VI. Other bacterial species as causal agents. To be published.

ECONOMIC ASPECTS OF POULTRY KEEPING IN CANADA[1]

II. Relationships Between Prices of Feed and Eggs and Their Effect on the Return to the Poultryman for Labour and Management

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(Received for publication May 31, 1941)

The purpose of the following analyses is to arrive at reasonably safe estimates of the income that poultry keeping may provide. For this purpose are presented: (1) indices of the monetary value to the poultry industry of various feeding stuffs as determined by their biological value to poultry; (2) the normal relationship between prices of eggs and of various feeding stuffs; (3) the normal relationship between egg prices through the seasons and throughout Canada; and (4) estimates of the poultryman's return for labour and management.

1. BIO-ECONOMIC EVALUATION OF VARIOUS FEEDING STUFFS FOR POULTRY

On the basis of the two quotations, $23.00 per ton for barley and $55.00 per ton for meat meal, it was found by the use of Stothart's (4) adaptation of Petersen's (5) formula that the value of 1 pound of digestible crude protein was 4.72 cents and of 1 pound of total digestible non-protein matter was 1.167 cents. In the final calculations of the indices of feeding stuff values presented in Table 1, digestibility coefficients for poultry by Axelson (1) were employed.

Table 1.—Indices of the monetary value to the poultry industry of various feeding stuffs on the basis of their biological value to poultry

Barley	0.9156	Soybean oil meal	1.7353
Buckwheat	0.8336	Fish meal	2.5438
Corn	0.9351	Meat meal	2.1895
Oats	0.7221	Bone meal	0.8193
Oats (hulled)	1.0574	Buttermilk (fluid)	0.1716
Wheat	1.0000	Commercial mixed feed 50/50 mash and scratch	1.0748
Wheat bran	0.7070		
Wheat middlings	0.6417		

The index quoted for commercially mixed feed does not take any account of milling, transportation and distribution charges. The retail price of this feed would probably be 25 to 30% higher than indicated by its nutritional value.

2. THE NORMAL RELATIONSHIP BETWEEN PRICES OF EGGS AND VARIOUS FEEDING STUFFS

The following analysis was based on observed relationships between the prices of eggs and of wheat. The yearly average Winnipeg quotations on No. 1 Northern wheat and the yearly average Toronto quotation on

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[2] Agricultural Assistant.

grade A large eggs as paid to producers were secured for the 27-year period from 1910 to 1936. The mean quotations were found to be 126.3 cents per bushel of wheat and 37.1 cents per dozen of eggs. The coefficient of correlation between the prices per bushel of wheat and per dozen of eggs was found to be: $r = + .895$ and the coefficient of regression proved to be: $b = 0.183$ cents per dozen of eggs for each 1 cent per bushel of wheat. Whenever mean yearly wheat prices, therefore, moved up or down one cent the mean yearly egg price moved 0.183 cents in the same direction.

This relationship is presumably an equitable one as it has held for 27 years, and withal poultry keepers are still in business. In order to test how the relationship has affected the economics of poultry keeping it is probably fair to assume that the average production for all Canadian hens through these 27 years has been around 8 dozen eggs per hen. An increase of 0.183 cents in the price per dozen eggs would give a total increase of 1.46 cents for the 8 dozen eggs. To produce these eggs a hen would consume something like 86 pounds of feed which, with an increase of 1 cent per bushel and assuming that all the feed was wheat, would give a total increase in the feed price to produce 8 dozen eggs of 1.43 cents. Even if many more factors would have to be taken into consideration for a complete analysis, the fit is close enough to show that the relationship between feed value of wheat and egg prices has been sound.

By virtue of the above mentioned relationships between prices of eggs and wheat the mean annual wheat prices as quoted in Winnipeg which would correspond to five different levels of mean annual egg prices as quoted in Toronto could be calculated. Using the information, which is presented in the following section, these five levels of Toronto egg prices were converted to mean annual Canadian egg prices and extended to show the relationships between Winnipeg wheat prices and mean annual egg prices as paid to producers for grade A large eggs in various representative locations in Canada. Applying the indices presented in Table 1, this information was further extended to show the prices on several other feeding stuffs corresponding to these various egg prices. The prices of these additional feeding stuffs are therefore determined by the relationship which exists between their biological value to poultry and that of wheat when the latter is quoted at Winnipeg prices. The prices quoted for commercially mixed feed represent as previously noted, its biological value only. These price relationships are presented in Table 2.

From this table it will be seen that when the annual mean Canadian price per dozen of eggs and the annual mean Winnipeg price per bushel of wheat are 25 cents and 73 cents, respectively, then eggs should fetch an annual mean price of 28.6 cents per dozen in Quebec City, and oats and fish meal would in relation to the Winnipeg wheat price have feeding values of 30 cents per bushel and 62 dollars per ton, respectively. The other columns of the table may be employed in similar manner when it is desired to obtain information regarding the relationship between annual mean egg prices and the annual mean feeding value of the listed materials in their relationship to Winnipeg wheat quotations.

TABLE 2

On five arbitrarily chosen levels of Canadian mean annual price per dozen of Grade A large eggs, as paid to producers, are presented the relationships between the same type of egg prices in representative locations across Canada and the value of various grains and feeds on the basis of mean annual wheat quotations at Winnipeg.

The relationship between prices of eggs and wheat is based on 27 years observations and the values of other grains and feeding stuffs are related to that of wheat according to their respective biological value to poultry.

Five arbitrary levels of Canadian annual mean egg price in cents per doz.	15.0	25.0	35.0	45.0	55.0
Corresponding annual mean Winnipeg wheat price in cents per bushel	29.3	73.2	117.1	161.0	204.9
Egg prices in cents per dozen					
Halifax, Saint John, Quebec City	17.1	28.6	40.0	51.4	62.8
Charlottetown	15.1	25.2	35.3	45.4	55.5
London, Ont.	14.5	24.2	33.9	43.5	53.2
Winnipeg	13.5	22.5	31.5	40.5	49.5
Regina, Edmonton	12.4	20.7	29.0	37.3	45.6
Vancouver	15.6	25.9	36.3	46.7	57.1
Grain prices in cents per bushel					
Barley	21.5	53.6	85.8	117.9	150.1
Buckwheat	19.5	48.8	78.1	107.4	136.6
Corn	27.4	68.5	109.5	150.5	191.6
Oats	12.0	30.0	47.9	65.9	83.8
Feeding stuff prices in dollars per ton					
Barley	8.95	22.34	35.74	49.14	62.54
Buckwheat	8.14	20.34	32.54	44.74	56.93
Corn	9.14	22.82	36.50	50.18	63.87
Oats	7.06	17.62	28.19	38.75	49.32
Oats (hulled)	10.33	25.81	41.27	56.74	72.22
Wheat	9.77	24.40	39.03	53.67	68.30
Wheat bran	6.91	17.25	27.60	37.94	48.29
Wheat middlings	6.27	15.66	25.05	34.44	43.83
Soy bean oil meal	16.95	42.34	67.73	93.13	118.52
Fish meal	24.85	62.07	99.29	136.52	173.74
Meat meal	21.39	53.42	85.46	117.50	149.54
Bone meal	8.00	19.99	31.98	43.97	55.96
Buttermilk (fluid)	1.68	4.19	6.70	9.21	11.72
Commercial 50/50 mash and scratch	10.50	26.23	41.95	57.68	73.41

3. THE NORMAL RELATIONSHIP BETWEEN SEASONAL EGG PRICES IN VARIOUS LOCATIONS

Wholesalers' quotations per dozen eggs of grade A large as paid to producers and country shippers were noted week by week through the years 1936-1939, inclusive (4), in nine representative locations across Canada. The four quotations thus available for each week and location were averaged, whereby nine local price curves through a full year were obtained, each curve composed of 52 weekly averages. The nine locations chosen as fairly representative of wider areas were Halifax, Charlottetown, Saint John, Quebec City, London (Ontario), Winnipeg, Regina, Edmonton and Vancouver.

For the purpose of making this survey of Canadian egg prices more generally useful, each one of the 468 weekly notations thus secured was expressed as a percentage of the general mean, which was found to be 23.989 cents. The following variance analysis of this datum revealed that highly significant differences existed between the prices in various weeks as well as between those in the various locations.

VARIANCE ANALYSIS

——	D.F.	Σ Sq.	Mean Sq.	F.	1% Point
Total	467	398796			
Between weeks	51	285764	5603.2	62.4	1.11
Between locations	8	77045	9630.6	109.2	2.56
Remainder	408	35987	88.2		

However, the necessary difference that must exist between prices in the individual locations to secure odds of 19 to 1 that such a difference did not occur by chance alone was calculated by the usual methods. See for instance Love (5, pp. 243-246). This gives a value of 3.61% for the necessary difference. As the difference between the means of the percentage prices quoted in Halifax, Saint John and Quebec City did not differ by this amount these three locations were regarded as having in fact

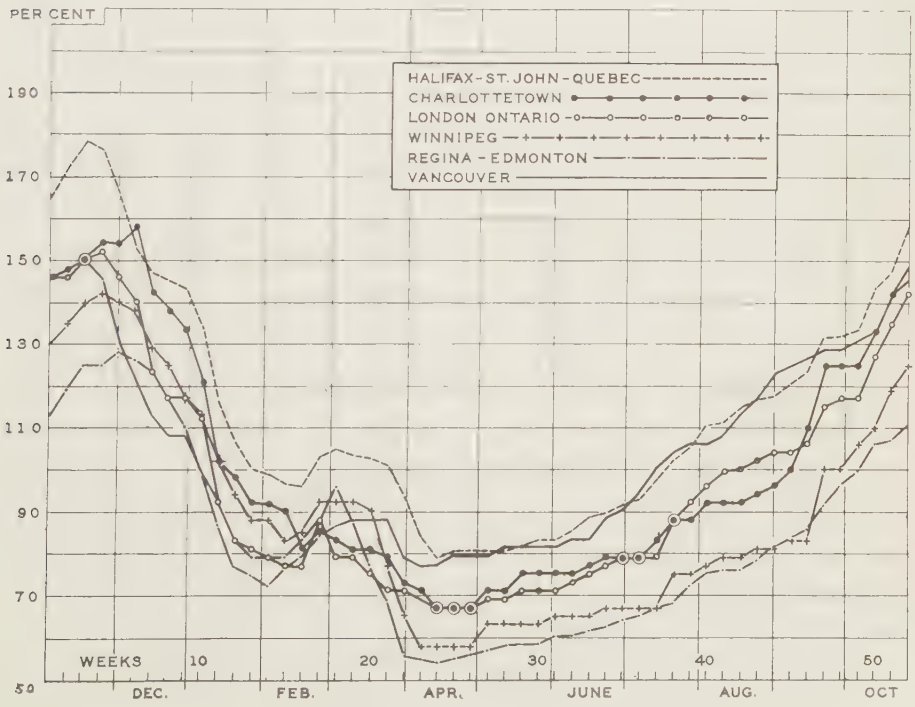

FIGURE 1. Four years average weekly egg prices in various locations across Canada expressed as percentage of the annual mean price for the Dominion.

TABLE 3.—LOCAL WHOLESALERS' QUOTATIONS ON GRADE A LARGE EGGS TO PRODUCERS AND COUNTRY SHIPPERS AS PERCENTAGES OF THE DOMINION MEAN QUOTATION FOR THE FOUR YEARS 1936-1939, INCLUSIVE

No. Week	Halifax, Saint John, Quebec	Charlotte-town	London	Winnipeg	Regina, Edmonton	Vancouver
1st Fri. in Nov.	164.7	146	146	129	112.5	146
2	172.3	148	146	135	119.0	148
3	178.3	150	150	140	125.0	150
4	176.3	154	152	142	125.0	146
5	166.0	154	146	140	128.0	131
6	153.3	158	140	138	126.0	121
7	147.3	142	123	129	123.0	113
8	145.3	138	117	125	116.5	108
9	143.3	133	117	117	110.5	108
10	134 0	121	102	113	105.5	100
11	115.3	102	92	102	86.5	92
12	106.0	98	83	94	77.0	83
13	100.0	92	81	88	75.0	79
14	98.7	92	79	88	72.0	79
15	96.7	90	77	83	76.0	79
16	96.0	81	77	85	79.0	83
17	103.3	85	88	92	84.0	88
18	104.7	83	79	92	86.5	96
19	103.3	81	79	92	88.0	88
20	102.7	81	75	90	77.0	88
21	100.7	79	71	77	69.0	88
22	93.3	73	71	65	56.0	79
23	84.0	71	69	58	55.0	77
24	79.0	67	67	58	54.0	77
25	80.3	67	67	58	55.0	79
26	80.3	67	67	58	56.0	79
27	80.3	71	69	63	57.0	79
28	80.3	71	69	63	58.5	81
29	81.7	75	71	63	58.5	81
30	83.0	75	71	63	58.5	81
31	83.0	75	71	65	60.5	81
32	85.3	75	73	65	60.5	83
33	88.3	77	75	65	61.5	83
34	89.7	77	79	67	62.5	88
35	91.3	79	79	67	64.0	90
36	92.7	79	79	67	65.0	94
37	97.3	83	79	67	67.0	100
38	102.0	88	88	75	68.0	104
39	105.3	92	88	75	72.0	106
40	110.3	96	92	77	75.0	106
41	111.0	100	92	79	76.0	108
42	114.3	100	92	79	76.0	113
43	116.3	102	94	81	78.0	117
44	117.7	104	96	81	81.5	123
45	120.3	104	100	83	83.5	125
46	123.7	106	110	83	85.5	127
47	131.7	115	125	100	91.0	129
48	132.0	117	125	100	96.0	129
49	133.3	117	125	106	99.0	131
50	143.3	127	133	110	106.0	133
51	148.0	135	142	119	107.0	142
52	159.7	142	150	125	110.5	146
Mean %	114.4	100.7	96.7	89.9	83.0	103.6
Mean price	27.4	24.2	23.2	21.6	19.9	24.9

the same price level. The same was found to be true for the prices quoted in Edmonton and Regina. In this way it was found that six percentage price curves will adequately present the egg prices paid by wholesalers to producers and country shippers for grade A large eggs across the Dominion. These six curves are presented in Figure 1 and Table 3.

It is noticeable that the six curves segregate into three definite and well defined groups from the beginning of April to the middle of September. Throughout the whole of this period supply and demand must exert their relative influence in a very nearly identical manner at all locations. During the remainder of the year local peculiarities in these respects evidently make for slight changes in the interrelationship of the curves. The main deviations from the general pattern are the relatively low winter prices quoted in Vancouver, the relatively high quotations in Charlottetown and the slightly different slope of the prairie curves.

Judiciously smoothed curves would probably make it possible to estimate future egg prices with very little error.

4. ESTIMATES OF THE POULTRYMAN'S RETURN FOR LABOUR AND MANAGEMENT

Unpublished data in this Division show that the average experience of mortality during five years on all Dominion Experimental Farms has been approximately 18% during the rearing period and likewise about 18% during the pullet laying year. These rates of mortality have therefore been applied in the following analyses.

Table 4 presents the cost of raising one pullet to sexual maturity or one cockerel to 6½ pound slaughter weight when 4000 baby chicks are purchased at $144.00 per 1000 and when all expenses are charged against the surviving birds.

TABLE 4.—COST OF RAISING ONE PULLET TO SEXUAL MATURITY OR ONE COCKEREL TO 6½ LB. SLAUGHTER WEIGHT (EXCLUSIVE OF OPERATOR'S RETURNS)

		c.
Purchase price per surviving chick		17.6
Feed—33 pounds per bird at $32.75 per ton for 82% surviving birds and ½ this amount for 18% that die, carried by 82% survivors		60.0
Heat and litter		2.2
Labour 6 months at $60.00		11.0
Depreciation—Buildings $2500.00 at 8%—$200.00		
Equipment 500.00 at 10%— 50.00		
Taxes—6 acres at $50.00 300.00	12.00	
Interest on investment $3300.00 at 5%— 165.00		
Total overhead	$427.00	
Overhead per surviving bird $427.00/3280		13.0
Total cost per bird		103.8

In this and the following estimates feed has been entered at $32.75 per ton. This represents the commercial value of mixed feed made up to 50%

mash and 50% scratch grain which corresponds to a price of 24 cents per dozen eggs which was the average price paid during four years throughout Canada by wholesalers to producers for grade A large eggs.

Table 4 shows that the cost of raising a pullet of the heavier breeds will amount to 103.8 cents. If a light breed is chosen the purchase price per surviving chick will be lower by 0.6 cents and feed cost lower by 14.6 cents with a total cost to sexual maturity of 88.6 cents.

Table 5 presents the cost of operating a poultry plant of 1500 laying birds which, for the purpose of the present analysis, is regarded as a suitable one man's full time labour proposition. The value and selling price of cockerels has not been included in this survey. At a rearing cost of 104 or 89 cents per bird with added expenses for dressing, dressing loss in weight and transportation, cockerels would probably just pay for themselves if sold at market quotations of 18 cents per pound.

In the following analysis it is assumed that an average flock is composed of 70% pullets and 30% yearling hens; further, that mortality and production are about evenly distributed around their respective modes which coincide in the spring or about the midpoint of the laying year. With 18% mortality such a flock would have, on the average 1500 less 9%, or 1365 producing units. The term "producing units" has in the following been used to denote the surviving birds with the addition of half of those that die. The expenses shown in Table 5 are therefore distributed over this number of potential profit earners. However, these expenses represent the bare maintenance cost only, the expenses entailed by egg production will be considered later.

TABLE 5.—TOTAL YEARLY MAINTENANCE COST FOR ONE PRODUCING UNIT (EXCLUSIVE OF OPERATOR'S RETURNS)

Maintenance feed for a 6-pound female 76.5 pounds* at $32.75 per ton				$1.25
Pullet renewal (.70 × 1500 × 103.8)/1365 producing units				.80
Litter				.12
Depreciation—Buildings	$3000.00	at 5%	$150.00	
Equipment	350.00	at 10%	35.00	
Taxes—2 acres at $50.00	100.00		4.00	
Dwelling	2000.00			
Stock	1550.00			
Supplies	200.00			
Interest on investment	$7200.00	at 5%	360.00	
Total overhead			$549.00	
Overhead per producing unit $549.00/1365				.40
Total maintenance cost per bird				$2.57

* Bird and Sinclair (3).

From Table 5 it will be seen that the cost of maintaining one producing unit of the heavier breeds through a laying year is 2.57 dollars. In cases where a light breed is chosen the feed cost will be 43 cents less according to Bird and Sinclair (3), and the pullet renewal will be 12 cents cheaper, making a total maintenance cost of 2.02 dollars.

TABLE 6.—ESTIMATE OF AMOUNT OF MEAT FOR DISPOSAL AT END OF EACH YEAR PER 100 BIRDS

Composition of flock		18% mortality number dead birds	Surviving number live birds	Carry over number birds	Disposal number birds
Yearling hens	30	5	25	—	25
Pullets	70	13	57	30	27
Total	100	18	82	30	52

In a survey of the probable revenue from a poultry enterprise it is of importance to estimate the amount of meat that may be available for marketing at the end of the year. Such an estimate is shown in Table 6 from which it may be seen that from a flock with a ratio of 70 pullets to 30 yearling hens and an experience of 18% mortality, 52% per cent will be available for marketing at the end of the year. Hence, on an average weight of six pounds, 52% per bird or 3·12 pounds will be available for market, and with an average weight for light breeds of 4 pounds per bird, 2.08 pounds will be available. When sold at 14 and 12 cents per pound for heavy and light breeds, respectively their cash value will be 44 cents and 25 cents, respectively for each bird that entered the laying year and 48 cents and 27 cents for each producing unit in the flock.

TABLE 7.—ESTIMATE OF THE POULTRYMAN'S RETURN FOR LABOUR AND MANAGEMENT AT FIVE LEVELS OF PRODUCTION

A

For a bird of heavy breed

Level of annual production number eggs	Cost of production			Receipts			Loss or gain
	Maintenance*	Feed cost for egg production	Total cost	Meat value	Sale of eggs	Total income	
	$	c.	$	c.	$	$	$
50	2.57	8	2.65	48	0.98	1.46	−1.19
90	2.57	14	2.71	48	1.76	2.24	− .47
130	2.57	21	2.78	48	2.54	3.02	+ .24
170	2.57	28	2.85	48	3.32	3.80	+ .95
210	2.57	34	2.91	48	4.10	4.58	+1.67

B

For a bird of light breed

50	2.02	8	2.10	27	0.98	1.25	− .85
90	2.02	14	2.16	27	1.76	2.03	− .13
130	2.02	21	2.23	27	2.54	2.81	+ .58
170	2.02	28	2.30	27	3.32	3.59	+1.29
210	2.02	34	2.36	27	4.10	4.37	+2.01

* Derived from Table 5.

It was shown in an earlier communication from this laboratory by Bird and Sinclair (2) that egg production per se proceeds at an average cost of .8753 gm. of feed per 1 gm. of edible content of eggs. Each 10 eggs will therefore cost approximately the value of 1 pound of feed to produce; at $32.75 per ton of feed this works out at 1.64 cents per 10 eggs. During the course of previous work it was found that an average flock produces 75% large, 20% medium and 5% pullet eggs. In the following estimates of the poultryman's returns for labour and management at various levels of production the three egg sizes have been valued at 24 cents, 22 cents and 20 cents per dozen.

The production levels in this table signify the mean production of all the producing units in a flock when mortality is at 18%. In order to maintain the gains or losses indicated for the five levels of production shown in Table 7 the mean production from the surviving birds would have to be 56, 100, 144, 189 and 233 eggs per bird.

These estimates indicate how relatively expensive it is to maintain a hen, and how ridiculously cheap it is to sustain production. The main reason why all hens do not lay at a remunerative level of production is probably lack of proper management. This term is here understood to cover proper feeding and housing, cleanliness, and freedom from parasites and disease.

The production levels at which expenses and income will balance leaving no returns for operator's labour and management, and also the levels at which the operator will receive 1 dollar gain from each producing unit and each surviving bird in a flock may be obtained by interpolation in Table 7 and are shown in Table 8.

Table 8.—Production levels per bird which yield

	No return from		$1 return from	
	Producing units	Surviving birds	Producing units	Surviving birds
Heavy breeds	116	129	173	192
Light breeds	97	108	154	171

From Tables 4 and 5 it will be seen that about $11,000 capital investment is required to set up a poultry enterprise of 1500 laying birds, which may be regarded as a full-time labour proposition for one man. In order that the net returns for operator's labour and management may represent 10 or 15% interest on the investment, respectively, the producing units and the surviving birds must give the average productions shown in Table 9.

The estimates of the poultryman's return for labour and management which have been presented herein are based on the Canadian mean egg price during four years and its corresponding feed price. The net result will probably not be altered to any great extent even if local mean prices, which may vary considerably from the Canadian means, are applied, inasmuch as such variations generally occur in the same direction for both eggs and feed.

TABLE 9.—PRODUCTION LEVELS PER BIRD WHICH YIELD 10 OR 15 PER CENT RETURN ON CAPITAL INVESTED

	10 per cent		15 per cent	
	Producing units	Surviving birds	Producing units	Surviving birds
Heavy breeds	160	178	182	202
Light breeds	141	156	162	180

For obvious reasons these estimates have not been concerned with the returns to be expected from poultry keeping in a general farm enterprise. However, it may be loosely estimated that under such conditions feed costs will be down to about two-thirds of the above estimates. Pullet renewal will be down to about one-half, because the purchase price of chicks, and labour and overhead are practically non-existent and other expenses very small. On the other hand revenue will be down on account of lower production and the bulk of production being obtained during the low price season, and lower quantity and quality of meat. The net result will therefore probably be that the levels of production which will yield no return and $1 return, respectively, per bird will be 25 to 30 eggs lower than those shown for a specialized poultry enterprise.

SUMMARY

Analyses have been presented showing the relationship in monetary value of various feeding stuffs as determined by their biological value to poultry. Also the normal relationship between mean annual prices of grade A large eggs, as paid to producers, and of various feeding stuffs has been presented.

The normal relationships in egg prices have been established throughout the year in representative locations in Canada. All Canadian egg prices are fairly represented by six price curves. There are significant differences between these curves.

Fundamental to estimates of costs and revenue has been the mean annual relationship of 24 cents per dozen A large eggs, as paid to producers, and 32.75 dollars per ton of mixed feed. On this basis rearing costs for one pullet to sexual maturity are 89 cents or 104 cents according to breed (exclusive of operator's returns). Costs (again exclusive of operator's returns) of maintaining one producing unit through the year will be 202 or 257 cents, respectively. Feed cost of egg production is about 7 cents for every 40 eggs. When 70% of the flock are pullets and mortality is 18%, 52% of the body weight of every bird that starts the year will be available for marketing at the end of the year. It is estimated that one dollar return per bird for operator's labour and management may be obtained when the surviving birds of heavy breeds produce at a yearly rate of 192 eggs and of light breeds at the rate of 171 eggs. The operator's return per

bird may represent 10% of the capital invested when surviving birds of heavy and light breeds produce at the rate of 178 and 156 eggs, respectively. The corresponding productions must be 202 and 180 eggs, respectively, if 15% is to be realized.

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THE PLACE OF CRESTED WHEAT GRASS, *AGROPYRON CRISTATUM, L.* IN CONTROLLING PERENNIAL WEEDS[1]

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Control of perennial weeds, such as quack grass, sow thistle, Canada thistle, poverty weed and toad flax, has long been a matter of considerable importance in Western Canada. In recent years the question of controlling perennial weeds has taken on new significance because of the spread of certain weeds which are known to relatively few farmers and which are exceptionally difficult to eradicate by tillage methods. These latter weeds include leafy spurge, *Euphorbia esula, L.*, hoary cress, *Cadaria draba, L., Desv.*, field bindweed, *Convolvulus arvensis, L.*, and Russian knapweed, *Centaurea picris, Pall.* Experimental tests have shown that black summerfallow, usually effective with quack grass, sow thistle and Canada thistle, is not completely effective with these weeds even when continued for two successive seasons. Apparently the roots are protected from decay by a strong corky bark which resists the attack of soil fungi and bacteria. While certain chemicals, such as sodium chlorate can be used to eradicate any of the perennial weeds, the treatment is too expensive for general use in cultivated fields, and the soil sterility which follows such treatment is very objectionable.

Whenever it is possible to control a weed by making it compete with a crop which is more aggressive than the weed and highly adapted to the habitat where the weed is found, such a method of control or eradication is usually not only the soundest procedure but the most economical as well. This is especially true of those perennial weeds which are exceptionally persistent and which require an abnormal amount of cultivation to effect their eradication. It is a sound principle of weed control to take advantage, wherever possible, of the relative competitive efficiency of weeds and crop plants. Thus barley is frequently used as a cleaning crop for the control of annual weeds because this cereal has a greater competitive efficiency than, for example, wheat.

For best results, perennial weeds require of course a perennial crop. Thus alfalfa is used extensively for the control of Canada thistle on irrigated land. It is essential that the competing crop shall be highly adapted to the environment. Such a crop in the prairie sections of Western Canada, is crested wheat grass. Experiments and practical usage have shown that no other grass has the competitive power of crested wheat grass in this area. It was decided therefore to investigate the effectiveness of this grass in the control of perennial weeds. In this experiment were included perennial sow thistle, Canada thistle, quack grass, toad flax, leafy spurge, hoary cress, field bindweed and poverty weed. The project was begun

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in 1933 on plots located at Saskatoon on good chocolate clay loam, the weeds being planted with root cuttings and the grass seeded two or three years later after the weeds had become well established.

The results obtained in these experiments have been sufficiently promising that more extensive tests are being laid down. In the meantime it is believed that a preliminary report may prove interesting and possibly induce other investigators to try similar experiments under a variety of conditions. Some of these weeds, notably leafy spurge, hoary cress, Russian knapweed and field bindweed are more widely distributed in the three Prairie Provinces than is generally realized and constitute a menace of serious proportions. Since the control measures that have been tried have not been very efficient from the standpoint either of effectiveness or cost of application, the use of crested wheat grass, if satisfactory, should be a welcome solution. Illustrations from 1 to 5 show the progress of competition between crested wheat grass and leafy spurge over a period of four growing seasons.

RESULTS

The results obtained thus far by the use of crested wheat grass may be stated briefly as follows:

1. In three seasons crested wheat grass has completely smothered thick stands of perennial sow thistle and toad flax.

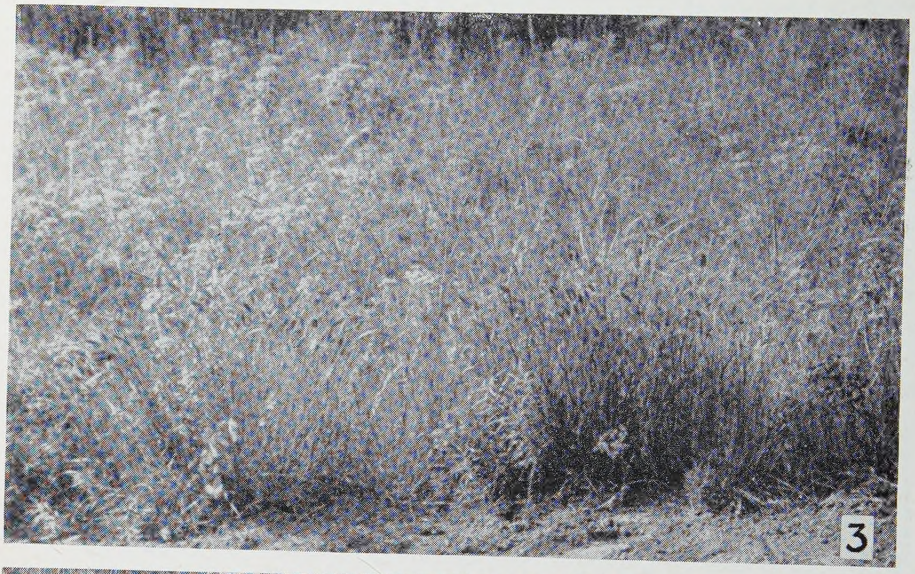
2. In four seasons it has eliminated a solid stand of Canada thistle with only a few suppressed weeds surviving in a depression where the soil contained more alkali salts. At the end of the fifth year the grass stand was entirely free of weeds.

3. Crested wheat grass sown in the fall into a solid stand of quack grass after the top growth had been closely mowed, and the surface scratched by a harrow heavily loaded with stones, nearly exterminated the weed in four years.

4. After four years of competition between crested wheat grass on the one hand, and leafy spurge, poverty weed, field bindweed, and hoary cress on the other, the weeds were so reduced in size that a hay crop cut from these plots was reasonably free of them. Close to the ground, however, many badly suppressed weeds were still present.

5. From these tests it might be said that the plant population of poverty weed, though considerably reduced as compared with the original stand, remained quite high; that of the field bindweed proportionally decreased much further. The plants of leafy spurge became scarce, while those of hoary cress were found mainly at the boarders of the plot but were virtually absent where the stand of grass was uniformly solid.

Illustrations in PLATES I and II were obtained from the plots of leafy spurge seeded to crested wheat grass. 1. A thick stand of the weed into which the grass was sown. 2. Second season of growth after the grass was seeded. 3. Third season. 4. End of June of the fourth season. 5. End of the fourth season of growth. Note gradual decrease of leafy spurge and progressive occupation of the plot by crested wheat grass.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